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STUDIES ON
THE BIOLOGY OF PENAEIDS

V. GOPALAKRISHNAN

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Thesis submitted to the University of Madras for the Degree of Doctor of Philosophy

BY

V. GOPALAKRISHNAN, M.A.

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PREFACE

The work was carried out in the University Zoological Research Laboratory, Madras, for the past four years and the author wishes to express his grateful thanks to Dr. C. P. Gnanamuthu, Director of the laboratory, for suggesting the problem, criticism and guidance throughout the course of the work, and to Dr. G. Krishnan, Reader in Zoology, for his valuable suggestions, constant help and making the necessary corrections in the manuscript. Thanks are due to Dr. D. V. Rajalakshman, University of Madras, for helping in the statistical treatment of the experimental results, and to Mr. R. K. Murthy, for assisting in the biochemical technique. The author is indebted to Dr. Laurence Irving, Alaska Health Centre, to Dr. George L. Clarke of Harvard University, and to Dr. Erichsen Jones of University of Wales, for their helpful suggestions and encouraging criticism. Thanks are also due to the Syndicate of the University of Madras for awarding a research studentship and to the Ministry of Education, Government of India, for awarding a research scholarship.

V. Gopalakrishnan

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PART I

THE PHYSIOLOGY OF DIGESTION IN *PENAEUS INDICUS* M. ED.

I. THE PHYSIOLOGY OF DIGESTION IN *PENAEUS INDICUS*

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THE PHYSIOLOGY OF DIGESTION IN PENAEUS INDICUS

Introduction

Although the physiology of digestion in a number of decapod crustaceans has been studied (See Roaf, 1908 b; Yonge, 1924, 1931, 1937; Vonk, 1937), comparatively little attention has so far been paid to this aspect of study in the prawns. Further, earlier work on digestion in Crustacea was mostly on animals living in temperate regions so that it was felt that an investigation of the anatomy and histology of the alimentary canal together with the digestive process in a tropical prawn will be of considerable interest in itself, as well as for purposes of comparison with the results obtained by other workers. It may also be mentioned that our knowledge of the digestive process in Indian types of Crustacea is confined to the crab *Paratelphusa hydrodromus* (Reddy, 1937) and two isopods, *Ligia exotica* and *Armadillio elevatus* (Chandy, 1938).

The earlier studies on the digestion of decapods show that the hepatopancreatic extract contains a number of enzymes which are able to digest different food substances (See Yonge, 1931, 1937; Vonk, 1937). One of the earliest workers to study the digestive enzymes of the crab was Hoppe-Seyler (1876), who showed that the hepatopancreatic secretion contains proteolytic (resembling trypsin), amylolytic and lipolytic ferments. Krukerberg (1879) demonstrated the presence of both tryptic and peptic ferments in the digestive gland. Later Frenzel (1884) also showed the presence of ferments identical with trypsin. The digestive gland of *Astacus fluviatilis* was found to possess a trypsin-like proteolytic ferment and also lipolytic and amylolytic ferments (Jordan, 1904). The studies of Roaf (1906, 1908 b) on the digestive gland in different Crustacea revealed that extracts of the gland digest starch, glycogen, maltose, sucrose, lactose, fibrin and methyl acetate, and also that both peptolysis and proteolysis occur. Giaja (1907, 1909—quoted by Yonge, 1931) showed the presence of enzymes capable of digesting carbohydrates and glucosides in different marine decapods. The properties of the proteolytic enzymes of the crabs *Cancer* and *Maja* have been described by Sellier (1910). The investigations of Yonge (1924) indicated that an extract of the

hepatopancreas of the Norway lobster *Nephrops norvegicus* digests starch, glycogen, sucrose, maltose and lactose, the optimum action of the enzyme being in a neutral medium. He also demonstrated that the extract splits up fat and esters and that it digests proteins, the activity being very strong in alkaline media. The p^H optima for the action of the digestive juice of *Astacus* on proteins, peptone etc., have been studied by Shinoda (1928). He showed the presence of an autolytic enzyme and also a little of erepsin. Kruger and Graetz (1927 a, 1928) however, failed to detect the presence of any autolytic enzyme in this animal and were able to find only an erepsin. This view has been supported by Kruger's (1929) later studies also. The p^H optimum for the action of lipase found in *Astacus* has been worked out separately by Kruger and Graetz (1927 b). Wiersma and Van der Veen (1928) showed the presence of an amylase, a maltase and an invertase in the same animal and also determined the p^H optima for their action. The gastric secretion of *Uca* has been found to digest fibrin (in an alkaline medium) and connective tissue (Verwey, 1930). This secretion contains a lipase, an amylase, and an invertase also. The properties of the proteolytic enzymes of *Maja squinado* has been studied in detail by Mansour-Bek (1932) and the existence of different proteases in the hepatopancreatic extract was indicated. A study of the physiology of digestion in the Indian crab *Paratelphusa hydrodromus* (Reddy, 1937) showed that the digestive gland contains an amylolytic enzyme, having optimal action in a neutral medium, a cytase, a proteolytic enzyme and a lipolytic enzyme. Recently Saitoo and Hino (1942) have made studies on the specificity of the enzyme present in the hepatopancreas of *Neptunus trituberculatus*.

Studies on the general anatomy of the alimentary canal in decapods have not been detailed here, but mention may be made of Patwardhan's (1937) memoir on *Palaemon* in which the anatomy and histology of the alimentary canal are described. Earlier workers like Jordan (1904), Williams (1907) and Yonge (1924) have paid special attention to the stomach of decapod Crustacea, and it has been shown that in forms like *Astacus, Homarus* and *Nephrops*, unlike in other groups of Crustacea, there is a powerful armature formed of calcified ossicles situated in the 'cardiac' fore-gut, which have several functions like giving attachment to the muscles, supporting the wall of the fore-gut and forming a powerful grinding apparatus. The movements of the ossicles are made possible by means of the muscles of the fore-gut, which are divided into two kinds,

the extrinsic muscles inserted on to ossicles lying in the walls of the fore-gut and the intrinsic muscles attached at both ends to other ossicles which also lie in the walls of the fore-gut (See Patwardhan, 1935). It has been shown that the gastric mill in decapods conforms to a typical plan, common to all species, the variations being due to difference in size and shape, coalescence, reduction or disappearance of one or more ossicles, and that the various types of gastric mills found in decapods can be arranged in a series showing a graded increase in complexity (Reddy, 1935; Patwardhan, 1935). The reduction of the gastric mill in penaeids is probably due to the quality of the food materials and also the habitat of the animal (Patwardhan, loc. cit.). The gastric mill of *Penaeus indicus* is significant in the presence of the oesophageal armature outside the gastric region and the supra-lateral teeth along the lateral folds of the stomach (Reddy, 1935; Patwardhan, 1935).

In the present study a description of the anatomy and histology of the digestive tract of *Penaeus indicus* is given, as this is felt necessary in studying the physiology of digestion. The latter study includes the localisation, specificity and properties of the different hydrolytic enzymes found in the animal and also the range and process of absorption of digested food materials.

Material and Methods

The material for the present study was collected from the Adyar backwaters of Madras. Panikkar and Aiyar (1937) have described in detail the ecology of this area. For a greater part of the year, the river is separated from the sea by a sand bar and only during the rainy season it opens into the sea. *Penaeus indicus* collected from this area are of comparatively larger size than those available in other brackish water areas of Madras and were preferred on account of their suitability for the present study.

Aqueous extracts of the hepatopancreas were made from a number of glands ground well with the required volume of distilled water. In all experiments the extracts were made to a strength of 20%, following Yonge (1924) and Reddy (1937). Toluol was used as an antiseptic in the study of carbohydrate digestion and hydrocyanic acid in the studies on protein and fat digestion (Yonge, loc. cit.).

Estimations of glucose were made using Benedict's quantitative reagent. For each experiment, the sugar reagent was

taken in a small conical flask with a narrow neck and to this was added a fixed volume of sodium carbonate and a few small pieces of clean porcelain to avoid bumping. The solution was then heated over a small flame burning under a wire gauze. The digestive mixture was taken in a graduated pipette and was poured drop by drop into the boiling Benedict's solution. During this titration, care was taken to keep the pipette in a slanting position and to avoid any disturbance to the flask, so as to ensure accuracy in the results obtained. At first the digests were added rapidly, but after the solution had become cloudy, the operation was slowed down and continued till there was no trace of blue colour in the solution.

Barfoed's reagent was used for detecting the reduction of disaccharides (Roaf, 1908 a; Yonge, 1924; Reddy, 1937). For each experiment, five ml. of Barfoed's reagent (Bodansky and Fay, 1947; Morrow, 1935) was taken in a test tube and one ml. of the digest added to it. Then the tube was placed in a water bath at a temperature of 40°C. and at the end of five and ten minutes the observations were made (Morrow, loc. cit.).

The products of protein digestion were estimated by Sorensen's formal-titration method (Bodansky and Fay, 1947). The products of digestion of fat were estimated by the direct titration of the fatty acids formed with sodium hydroxide solution, using phenolphthalein as indicator.

For all experiments other than glycogen and fat digestion, the buffer mixtures of McIlvaine (Clark, 1922) and Atkins and Pantin (1926) were used. For glycogen digestion, the buffer mixture of Northrop (1922) as modified by Nicol (1931), and for fat digestion, the method adopted by Nicol (loc. cit.) were adopted.

Controls were set up for all experiments, using boiled extract. Duplicate estimations were made in all experiments.

For histological preparations of the alimentary canal, the prawns were dissected in normal saline and the tissues transferred to suitable fixing fluids such as Bouin-Duboscq, Mercuric chloride-formal, Carnoy's, Corrosive sublimate-acetic and Formalin. Best results were obtained by staining the sections in Heidenhain's Iron haematoxylin counterstained with eosin or orange-G. For decalcification, material fixed in Bouin's fluid was treated with 2% nitric acid alcohol for twentyfour to forty-eight hours. Frozen sections were prepared from tissue fixed in 10% formalin for over twentyfour hours. To study the fat

present, the sections were stained in Scarlet Red or Sudan III and differentiated in alum haematoxylin.

The Alimentary Canal

In *Penaeus indicus*, as in other decapod crustaceans, the alimentary canal shows division into three regions, the fore-gut, the mid-gut and the hind-gut. The mouth is a moderately big opening on the ventral surface of the cephalic region, and is bound by portions of the mouth parts as in *Palaemon* (Patwardhan, 1937). Leading from the mouth is the region termed as "buccal cavity," where the mandibular processes, which by their opposing dental plates serve to crush the food materials, are seen projecting.

The oesophagus is a very short tube running vertically dorsalwards at right angles to the long axis of the animal. In the arrangement of the cell layers, it resembles the oesophagus of *Palaemon* (Patwardhan, 1937). The oesophageal walls of *Penaeus* have four thick muscular longitudinal strands projecting into the lumen, separated from each other by grooves on the inside. The inner walls of the strands are lined by cuticle as in allied decapods (Yonge, 1932; Richards, 1951). This cuticular layer is about 25 μ in width, consisting of an epicuticle (Fig. 1, ep. c.) and an endocuticle (en. c.), the latter with prominent longitudinal striations. Underlying the cuticle is a layer of epithelial cells (e. c.), formed of columnar cells, about 30 μ in length and 4 μ wide. Below the layer of epithelial cells is the basement membrane (b. m.) and the connective tissue layer (c. t.) which consists of a reticular mass of tissue. Next to the basement membrane and in the connective tissue are present the circular and longitudinal muscle fibres (c. m. & l. m.). The circular muscles are the constrictor muscles. In addition to these two kinds of muscle fibres, dilator muscle fibres connected to the cuticular layer are also found. The tegumental glands (t. g.) are found in the connective tissue beneath the epithelial cells. The gland is globular measuring about 10 μ in diameter, formed of conical cells with their apices directed towards the outer side. The secretion from the cells is poured into a cavity connected with the lumen of the oesophagus by a long, narrow duct (t. g. d.) passing between the epithelial cells. The tegumental glands in the fore-gut of decapods have been described in detail by Balss (1927) and Yonge (1932).

The oesophagus leads into the stomach, which as has already been described by Reddy (1935) and Patwardhan

(1935) shows two divisions, the 'cardiac' and 'pyloric' regions. Though these two terms do not have the correct significance with regard to the position of the heart, they are retained here for convenience following earlier authors. At the region of the opening of the oesophagus into the cardiac stomach are present six sharp spines (Fig. 2, oe. t.) called the oesophageal teeth (Reddy, 1935). The histology of the stomach wall is essentially the same as that of the oesophagus, the epithelial cells (Fig. 3, e. c.) being about 40 μ long. The connective tissue layer (c. t.) shows the presence of circular and longitudinal muscle fibres (c. m. & l. m.). As in the oesophagus the epithelium of the 'cardiac' stomach has a thick cuticular lining (Fig. 3, ep. c. & en. c.) which at certain regions gets thickened into ossicles to form the gastric mill.

As in *Palaemon*, the 'cardiac' stomach of *Penaeus indicus* extends considerably and forms a broad chamber with a narrow floor. On either side of the floor are a longitudinal groove and a lateral fold the latter with a row of sharp denticles termed supra-lateral teeth by Reddy (1935). The mesocardiac ossicle (Fig. 2, m. c. o.) is a median ossicle on the dorsal wall of the 'cardiac' fore-gut and has its apex pointing forwards as described by Patwardhan (1935) and is fused posteriorly to the anterior end of the urocardiac ossicle (u.c.o.). The posterior end of the plate-like urocardiac ossicle projects into the chamber as a median tooth. On this median tooth are present lateral denticles also. Attached to the base of the mesocardiac ossicle are present the pterocardiac ossicles (p.c.o.). The two zygocardiac ossicles (z.c.o.) which are attached to the pterocardiac ossicles anteriorly and pyloric ossicle (p.o.) posteriorly, have in each a single median tooth and a row of curved small denticles projecting into the cavity of the 'cardiac' stomach. A small triangular propyloric (p.p.o.) ossicle which is connected to the pyloric ossicle is present as shown by Patwardhan (1935).

A valve like arrangement formed by a flap with long setae, separates the 'cardiac' and 'pyloric' regions of the stomach. The lateral walls of the 'pyloric' portion are considerably thickened and divide the cavity into two regions, the dorsal and ventral. The lateral walls and floor of the ventral division have thick cuticular linings internally, which constitute the characteristic filtering apparatus (Fig. 2, f.a.) (Reddy, 1935).

The opening of the fore-gut into the mid-gut is provided with a single median and two pairs of lateral valves, together

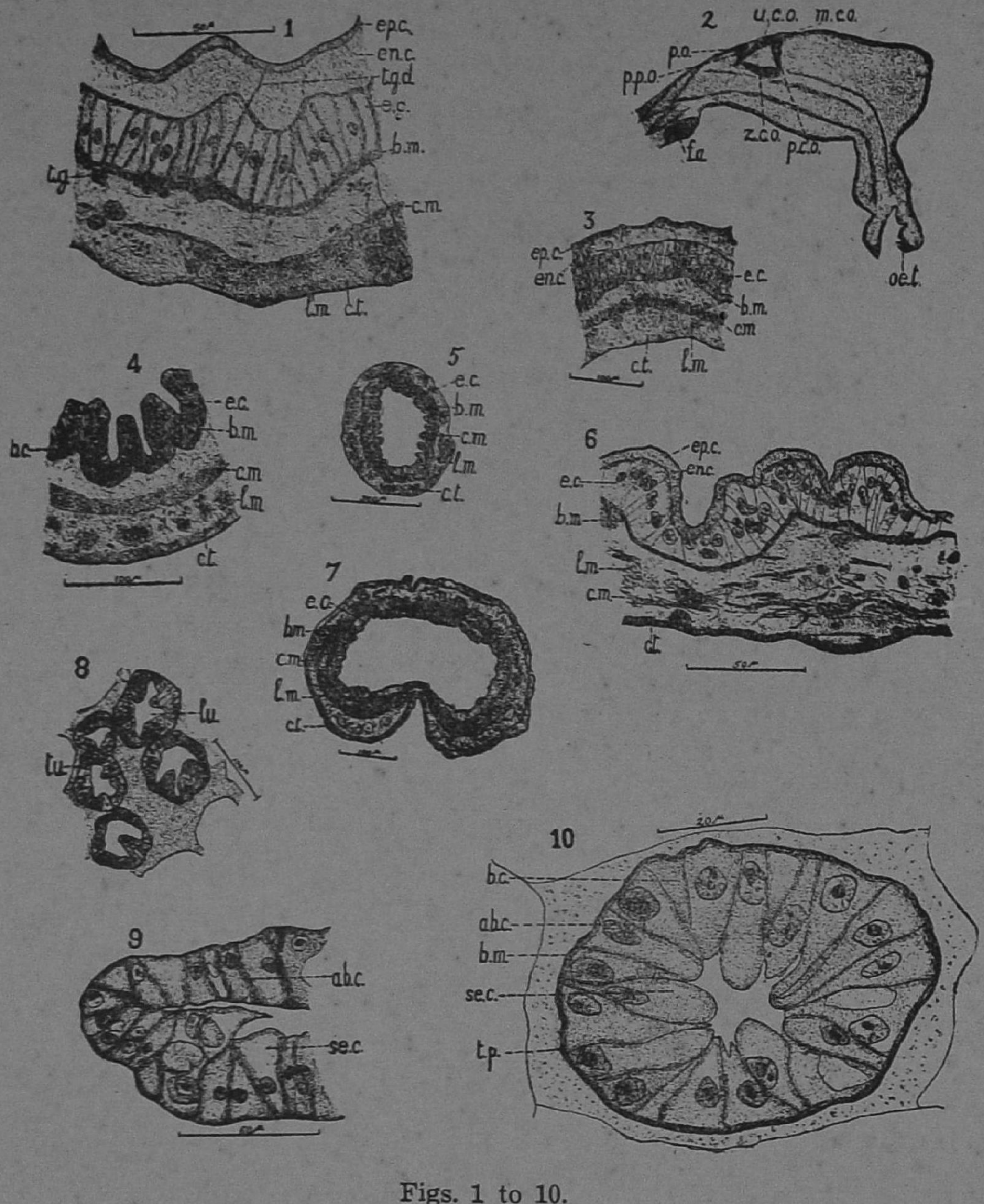
forming a sieve like arrangement. The mid-gut is a long and narrow tube with slender walls, stretching from the thoracic region to almost the entire length of the abdomen. The structure of the mid-gut wall is similar to that in *Palaemon* (Patwardhan, 1937). There is no cuticular lining in this region of the digestive tract. The epithelium is formed of columnar cells (Fig. 4, e.c.) of length 30 to 40 μ and containing refractive bodies. Basal or replacing cells (b.c.) are present as in the mid-gut of other decapods. The epithelium of the mid-gut is thrown into villi-like longitudinal folds which are more pronounced in the anterior region than in the posterior region. Beneath the layer of epithelial cells is the basement membrane (b.m.) 6 μ thick and then the circular muscles (c.m.), longitudinal muscles (l.m.) and connective tissue (c.t.).

The hind-gut is a very short tube with thick muscular walls, connecting the mid-gut and the anus. The cuticular lining of this region is thrown into folds and consists of the outer layer (Fig. 6, ep.c.) and the inner pigmented layer (en.c.), both together measuring about 6 μ in thickness. The epithelium is formed of elongated cells (e.c.), about 30 μ in length, with large nuclei. The epithelial layer of the hind-gut shows only small longitudinal folds projecting into the lumen (Fig. 7). The next layer is the basement membrane (Fig. 6, b.m.) followed by the circular muscles (c.m.), longitudinal muscles (l.m.) and connective tissue coat (c.t.).

The anus is a slit-like opening, the walls of which possess extensions of the longitudinal muscle fibres of the hind-gut. In addition, the edges of the opening are provided with radiating muscle fibres.

The hepatopancreas (Digestive gland)

The digestive gland of *Penaeus indicus* occupies the major portion of the cephalothoracic cavity and is made up of loosely arranged hepatopancreatic tubules (Figs. 8, 9), each tubule in cross section showing a characteristic four rayed shape. The lobulations are called 'acini' and each 'acinus' has a lumen in the centre. The lumen of different acini join together to form the duct of the hepatopancreas. The thin external layer of connective tissue (Fig. 10, t.p.), the tunica propria, incorporates the circular and longitudinal muscle fibres and also the basement membrane (b.m.). The arrangement of the muscle fibres in the hepatopancreas of decapods has been studied in detail by Pump (1914). The epithelial cells are either secretory or



Figs. 1 to 10.

EXPLANATION OF FIGURES

Fig. 1. Portion of wall of the oesophagus. Transverse section.

„ 2. Median section of the stomach showing the position of the various ossicles. (Semi-diagramatic).

„ 3. Portion of the wall of the cardiac stomach. Transverse section.

Fig. 4. Portion of the wall of the mid-gut. Transverse section.

" 5. Transverse section through the mid-gut.

" 6. Portion of the wall of the hind-gut. Transverse section.

" 7. Transverse section through the hind-gut.

" 8. Transverse section through the hepatopancreas.

" 9. Longitudinal section through a tubule of the hepatopancreas.

" 10. Transverse section through a tubule of the hepatopancreas.

ABBREVIATIONS

ab.c.	Absorptive cell.	oe.t.	Oesophageal teeth.
b.c.	Basal cell.	p.c.o.	Pterocardiac ossicle.
b.m.	Basement membrane.	p.o.	Pyloric ossicle.
c.m.	Circular muscles.	p.p.o.	Propyloric ossicle.
c.t.	Connective tissue.	se.c.	Secretory cell.
e.c.	Epithelial cell.	t.g.	Tegumental gland.
en.c.	Endocuticle.	t.g.d.	Duct of tegumental gland.
ep.c.	Epicuticle.	t.p.	Tunica propria.
f.a.	Filtering apparatus.	tu.	Tubule.
l.m.	Longitudinal muscles.	u.c.o.	Urocardiac ossicle.
l.u.	Lumen.	z.c.o.	Zygocardiac ossicle.
m.c.o.	Mesocardiac ossicle.		

absorptive in function. The secretory cells (se.c.) resemble the ferment cells present in the hepatopancreas of *Cancer* (Pearson, 1908). Each cell has a club-shaped appearance and is filled with vacuoles containing the secretion. All the cells have striated margins and the nuclei are placed at the narrow basal region of the cells. These cells measure about 30 to 40 μ in length and 10 to 20 μ in width. The absorptive cells (a.b.c.) resemble the 'fat cells' of *Cancer* (Pearson, 1908) and the hepatic cells of *Palaemon* (Patwardhan, 1937) and *Cambarus* (Dorman, 1928). Each cell measures about 25 to 40 μ in length and 5 to 15 μ in width and is of the columnar type with nuclei at the

basal regions and with the inner margins striated. The secretory cells are seen to occur in the middle region of the inwardly projecting parts of the epithelium and the absorptive cells occur between them. The arrangement of these cells appear to vary in different decapods, as may be inferred from the occurrence of these two types of cells side by side in *Paratelphusa* (Reddy, 1937). In addition to these secretory and absorptive cells, small basal or replacing cells (b.c.) are also present, lying near the basement membrane. Blood spaces occur between the tubules of the hepatopancreas.

Passage of food materials through the gut

In order to study the passage of food materials through the alimentary canal, two methods of observation were attempted. The first was to feed the prawns kept in the laboratory tanks with food material stained in methylene blue or carmine (Reddy, 1937) and to examine the progress of the food in the live condition. The other method was to kill the experimentally fed animals after different intervals of time and to dissect out the gut for examination under the binocular microscope.

Food material chopped up by the mouth parts are first passed into the oesophagus. The oesophageal movements are caused by the action of the constrictor and dilator muscles and the movements are similar to those described in *Nephrops* (Yonge, 1924). Thorough mastication of the material takes place in the cardiac fore-gut as in other decapods (Yonge, loc. cit.; Reddy, 1937). The regular movements of the gastric armature are clearly visible in a live prawn. The digested food materials pass into the pyloric fore-gut and eventually into the mid-gut. The hepatopancreas of the experimentally fed animals, when dissected after twentyfour hours show the presence of the stain used (Yonge, 1924), which evidently is absorbed through the mid-gut epithelium. The peristaltic movements of the mid-gut walls pass the rest of the food materials into the hind-gut. Such movements have been described in detail by Yonge (1924) in *Nephrops* and Reddy (1937) in *Paratelphusa*. Reddy observed that perfusion with adrenalin and epinine enhanced the peristaltic movements of the mid-gut to a very great extent. It is thought that an extensive nerve plexus controls these peristaltic movements (Miller, 1910; Alexandrowicz, 1909; Yonge, 1924).

Hydrogen-ion concentration of the gut contents

The hydrogen-ion concentration of guts from twelve specimens of *Penaeus indicus* was determined by the spot method of Clark and Lubs. The freshly caught specimens were dissected and portions of gut contents, after mixing with a drop of distilled water, were tested with the indicator. It was observed from the readings obtained that the p^H values for all regions of the alimentary canal except the cardiac stomach were the same, the value being 7.2. The average p^H of contents of the cardiac stomach was 7.0.

Localisation of the digestive enzymes

With a view to locate the enzymes involved in the process of digestion in *Penaeus indicus,* water extracts from different regions of the alimentary canal were prepared. It was seen that the hepatopancreas gave evidence of the presence of a number of digestive enzymes, while extracts prepared from the other regions of the alimentary canal such as stomach, intestine and oesophagus gave only feeble reactions for the presence of enzymes. Such weak reactions do not have any significance in the localisation of the enzymes. The secretion of the hepatopancreas, as in other allied crustaceans, is brownish in colour and is poured into the region of the pyloric stomach through the hepatopancreatic ducts.

The digestion of carbohydrates

Experiments were set up to determine the range of activity of the carbohydrate digesting enzymes present in the hepatopancreatic extract of *Penaeus indicus.* In each experiment 10 ml. of the extract, 10 ml. of the carbohydrate solution, the buffer and toluol were taken together in a tube and incubated for a fixed period as shown in Table I. At the end of the period each digest was boiled, made up to the original volume and the degree of digestion was estimated. The results given in Table I show the activity of the extract towards different carbohydrates. Controls were set up for each experiment by incubating the starch solution with boiled extract. The difference between the volume required for titration in experiment and control represent the amount of digestive activity during the period for which the experiment was conducted. In those instances where reduction of disaccharides was expected, Barfoed's test was applied. It will be noted from the results obtained that the hepatopancreatic extract of *Penaeus indicus* digests starch,

TABLE I

THE ENZYME ACTIVITY ON CARBOHYDRATES*

Temperature of experiment, 32°C

p^H of experiment, 7·2

Control with boiled extract

Digestive mixture	Duration of experiment	Experiment vol. required for titration, in ml.	Control vol. required for titration, in ml.
		For 10 ml. Benedict's solution	
10 ml. extract and 10 ml. 2% starch solution	3 hrs.	(i) 13·9	22·75
		(ii) 13·3	22·70
		(iii) 13·1	22·60
		For 5 ml. Benedict's solution	
10 ml. extract and 10 ml. 2% sucrose solution	7 hrs.	(i) 7·4	16.9
		(ii) 7·8	17·1
		(iii) 7·2	17 3
10 ml. extract and 10 ml. 0·5% glycogen	7 hrs.	(i) 11·0	17.5
		(ii) 12·05	17·7
		(iii) 11·7	17·3
10 ml. extract and 10 ml. 1% inulin	7 hrs.	(i) 15·55	15·8
		(ii) 14 8	15·0
		(iii) 15·3	15·6
10 ml. extract and 10 ml. 1% raffinose	7 hrs.	(i) 15·1	14·9
		(ii) 15·4	15·5
10 ml. extract and 1 gm. saw dust	5 days	(i) 14 3	14·5
		(ii) 14·7	14·8
10 ml. extract and 10 ml. 5% gum arabic	5 days	(i) 17·75	18·0
		(ii) 18·6	18·5
		1 ml. boiled with Barfoed's reagent	
10 ml. extract and 10 ml. 2% maltose	48 hrs.	Reduction	No reduction
10 ml. extract and 10 ml. 2% lactose	48 hrs.	Reduction	No reduction

TABLE II

THE OPTIMUM p^H FOR STARCH DIGESTION

10 ml of extract, 10 ml. of 2% starch solution, N/20 NaOH or HCl and water.
Temperature of experiment, 32°C
Duration of experiment, 5 hrs.
For control identical mixtures with boiled extract
More than one volume of digestive mixture whenever necessary

Average p^H	Experiment vol. in ml. required for 10 ml. Benedict's solution	vol. in ml. required for 10 ml. Benedict's solution	Difference in ml.
6·2	16·8	21·7	4·9
6·6	16·4	21·8	5·4
7·0	12·4	21·7	9·3
7·2	12·4	21·8	9·4
7·6	15·2	21·7	6·5
7·8	16·5	21·7	5·2

sucrose, maltose, lactose and glycogen. There was no action on inulin, raffinose, saw dust (cellulose) and gum arabic (pentosans).

Influence of hydrogen-ion concentration on digestion of starch

Experiments were conducted to find out the influence, if any, of hydrogen-ion concentration on starch digestion. For each experiment 10 ml. of 2% starch solution, acid or alkali solution and toluol were incubated together in a test tube for a period of five hours. After boiling and making up to the original volume with distilled water, each was titrated to 10 ml. Benedict's solution. Rigid controls were set up for each experiment. The results obtained are presented in Table II. The hydrogen-ion concentration ranged from 6.2 to 7.8 in the p^H scale, and the optimum for the action of the enzyme was found to be between p^H 7.0 and 7.2. The activity of the enzyme in relation to the changes in hydrogen-ion concentration is represented graphically in Fig. 11.

Identical experiments were conducted to observe the influence of time on the optimum p^H for starch digestion. For these experiments the periods of incubation were increased to ten and

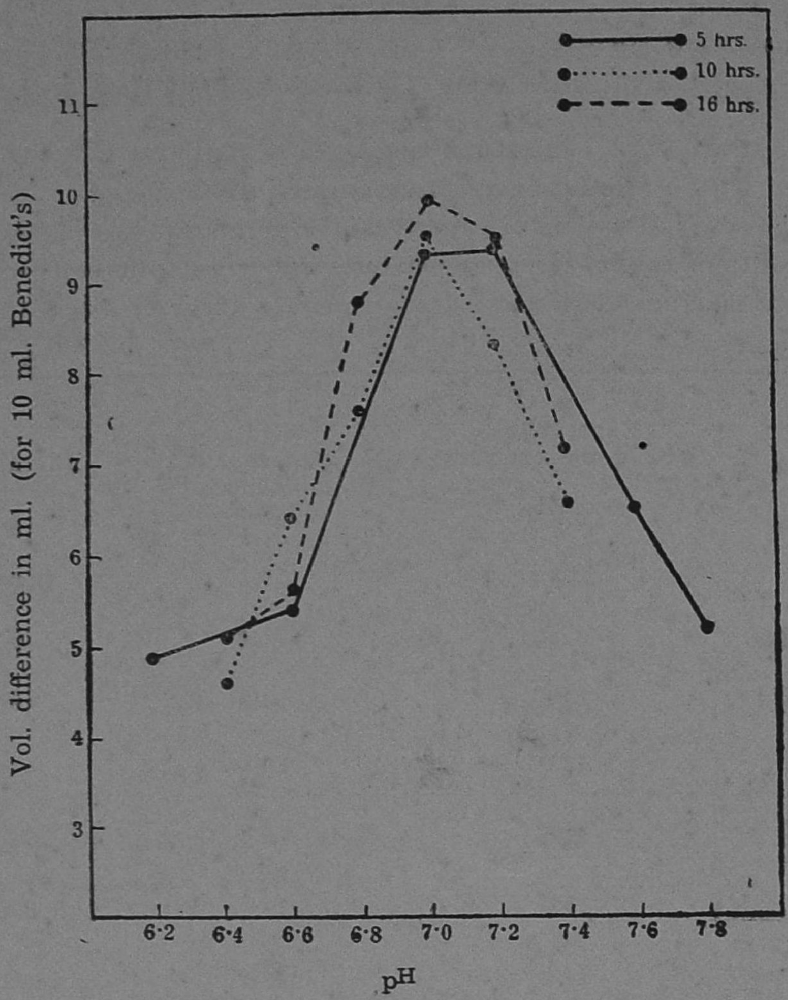


Fig. 11.

Curves illustrating the effect of pH on the digestion of starch and the influence of time on optimum pH.

sixteen hours respectively and the results obtained (Table III) show that the hydrogen-ion concentration at which digestion is optimum is not altered even when the period of incubation is increased. The results are represented in Fig. 11.

Influence of temperature on the optimum pH was also studied. The period of experiment was constant at five hours and the digests were incubated at temperature of 24°, 35° and 42°C. The results obtained (Table IV and Fig. 12) show that the variations in the temperature at which the experiment is performed do not affect the optimum pH for starch digestion.

Influence of temperature on the digestion of starch

Before determining the optimum temperature of the amylolytic enzyme of *Penaeus indicus*, preliminary tests were made

TABLE III

THE INFLUENCE OF TIME ON OPTIMUM p^H

10 ml. of extract, 10 ml. of 2% starch solution, N/20 NaOH or HCl and water.
Temperature of experiment, 32°C
Duration of experiment, 10 & 16 hrs.
For control indentical mixtures with boiled extract
More than one volume of digestive mixture whenever necessary

Average p^H	Experiment vol. in ml. required for 10 ml. Benedict's solution	Control vol. in ml. required for 10 ml. Benedict's solution	Difference in ml.
10 hrs.			
6·4	14·9	19·5	4·6
6·6	13·0	19·4	6·4
6·8	11·8	19·4	7·6
7·0	10·0	19·5	9·5
7·2	11·3	19·6	8·3
7·4	12·9	19·5	6·6
16 hrs.			
6·4	15·5	20·6	5·1
6·6	14·9	20·5	5·6
6·8	11·7	20·5	8·8
7·0	10·8	20·7	9·9
7·2	11·1	20·6	9·5
7·4	13·4	20·6	7·2

TABLE IV

INFLUENCE OF TEMPERATURE ON OPTIMUM p

10 ml. of extract, 10 ml. of 2% starch solution, N/20 NaOH or HCl and water
Duration of experiment, 5 hrs.
For control identical mixtures with boiled extract
More than one volume of digestive mixture whenever necessary
Temperature of experiment, 24, 35 & 42°C

Average p^H	Experiment vol. in ml. required for 10 ml. Benedict's solution	Control vol. in ml. required for 10 ml. Benedict's solution	Difference in ml.
Temperature, 24°C			
6·4	15·6	21·7	6·1
6·6	13·7	21·7	8·0
6·8	12·3	21·6	9·3
7·0	11·8	21·6	9·8
7·2	13·9	21·5	7·6
7·4	14·9	21·7	6·8
Temperature, 35°C			
6·4	15·1	22·2	7·15
6·6	14·1	22·4	8·3
6·8	12·6	22·3	9·7
7·0	12·1	22·4	10·3
7·2	13·4	22·3	8·9
7·4	14·5	22·3	7·8
Temperature, 42°C			
6·4	14·8	22·1	7·3
6·6	13·2	22·1	8·9
6·8	12·1	22·1	10·0
7·0	12·1	22·2	10·1
7·2	12·5	21·9	9·4
7·4	14·6	22·1	7·5

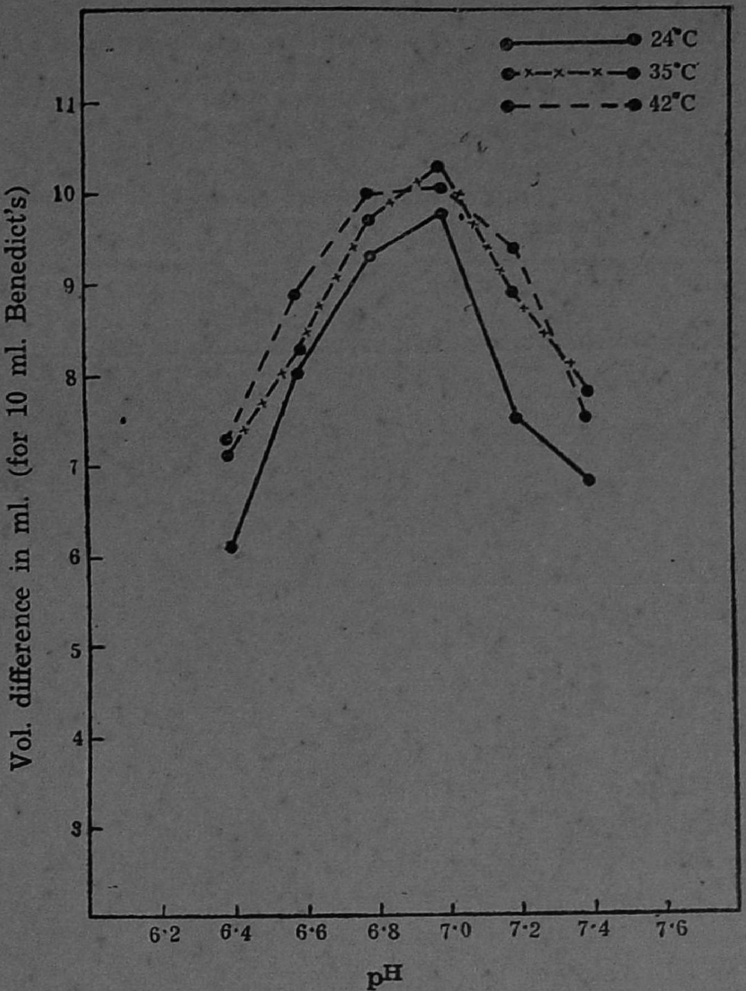


Fig. 12.

Curves illustrating the influence of temperature on optimum pH.

to have an idea of the range. The digests were incubated at temperatures varying from 40° to 61°C., and after four hours each sample was boiled, filtered and made up to the original volume. This was then titrated to 10 ml. Benedict's solution. The results obtained are given in Table V and Fig. 13. It will be noted that the optimum temperature for the enzyme action is about 52°C.

The temperature of destruction of the enzyme also was determined. Different samples of the extract were heated to various temperatures for fifteen minutes and mixed with starch solution as shown in Table VI. The results obtained show that the temperature of destruction of the enzyme is between 71° and 74°C. The progressive destruction of the amylase at higher temperatures is represented graphically in Fig. 14.

3

TABLE V

DETERMINATION OF THE OPTIMUM TEMPERATURE FOR STARCH DIGESTION

Duration of experiment, 4 hrs.

p^H of experiment, 7.2

Control with boiled extract

More than one volume of digestive mixture whenever necessary

Digestive mixture	Experiment vol. in ml. required for 10 ml. Benedict's solution	Control vol. in ml. required for 10 ml. Benedict's solution	Difference in ml.
10 ml. of extract and 10 ml. of 2% starch at 40°C	12·1	22·6	10·5
10 ml. of extract and 10 ml. of 2% starch at 43°C	10·9	22·5	11·6
10 ml. of extract and 10 ml. of 2% starch at 46°C	10·3	22·6	12·3
10 ml. of extract and 10 ml. of 2% starch at 49°C	9·8	22·4	12·6
10 ml. of extract and 10 ml. of 2% starch at 52°C	8·9	22·4	13·5
10 ml. of extract and 10 ml. of 2% starch at 55°C	10·5	22·6	12·1
10 ml. of extract and 10 ml. of 2% starch at 58°C	12·6	22·5	9·9
10 ml. of extract and 10 ml. of 2% starch at 61°C	15·4	22·5	7·1

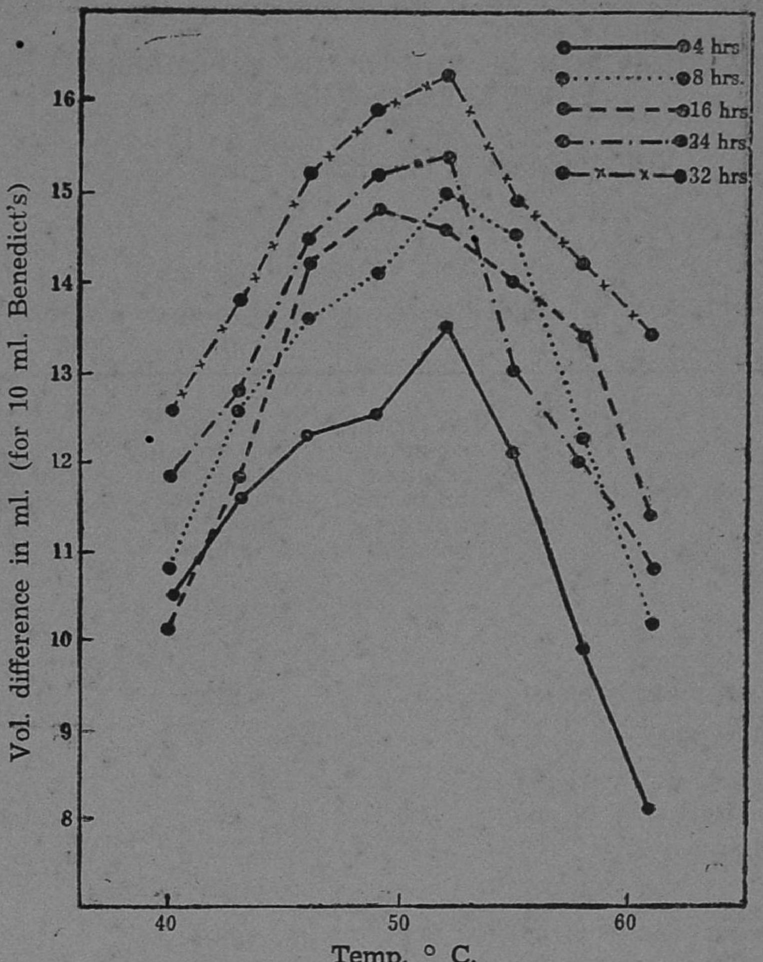


Fig. 13.

Curves illustrating the effect of temperature on the digestion of starch and the influence of time on temperature optimum.

The above results suggest that the influence of temperature on the activity of the digestive enzyme is purely a chemical phenomenon and has no biological significance, because the upper lethal temperature (See part II) limit of this prawn is about 43°C, while the temperature at which maximum enzyme activity takes place is about 52°C. Similarly the temperature of destruction also, being comparatively higher, has no relation to the normal digestive activity of the living prawn. Hence significant differences in the optimum temperature and temperature of destruction of the enzyme are not observed in forms living in tropical and non-tropical regions. The slight differences that have been recorded might be due to individual variations only rather than to changes in temperature of the habitat. Yonge (1924) observed that though the lobster *Nephrops* is a cold-blooded animal living in comparatively deeper waters, the temperature for the action of amylolytic enzyme is considerably

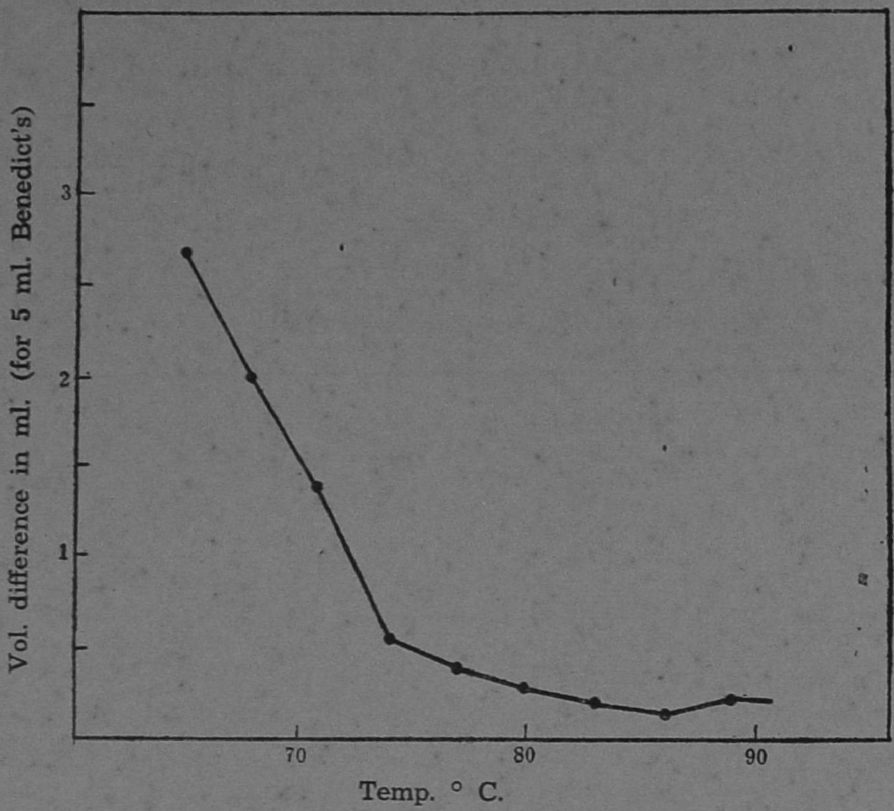


Fig. 14.

Curve illustrating the progressive destruction of the amylase at various temperatures after 15 minutes' heating.

higher than that of warm-blooded animals living in a much warmer environment. Eventhough *Penaeus* lives in conditions warmer than those for *Nephrops*, the optimum temperature and temperature of destruction of the enzyme are comparatively lower. The same is true of the crab *Paratelphusa* (Reddy, 1937). It may also be mentioned that according to Blackman (1905) the optimum temperature does not depend on any special property of the enzyme concerned.

With a view to study the influence of time factor on the optimum temperature, identical experiments were conducted after increasing the period to eight, sixteen, twentyfour and thirtytwo hours (Table VII). The temperature at which the digests were set up were the same as before, viz., 40, 43, 46, 49, 52, 55, 58 and 61°C. The results obtained show that the optimum temperature remains the same even after prolonged digestion (Fig. 13). A similar observation has been made by Nicol (1931) while studying the properties of the enzyme of the polychaete *Sabella pavonina*. However, Blackman (1905) and Berril (1929) have obtained results contrary to the above ob-

TABLE VI

DETERMINATION OF TEMPERATURE OF DESTRUCTION OF AMYLASE

10 ml. of extract heated for fifteen minutes at various temperatures and
10 ml. of 2% starch solution
Duration of experiment, 4 hrs.
p^H of experiment, 7·2
Control with boiled extract
Incubated at 32°C

Temperature °C	Experiment vol. in ml. required for 5 ml. Benedict's solution	Control vol. in ml. required for 5 ml. Benedict's solution	Difference in ml.
65	8·7	11·4	2·7
68	9·3	11·3	2·0
71	9·9	11·3	1·4
74	10·7	11·25	0·55
77	10·8	11·2	0·4
80	11·0	11·3	0·3
83	11·0	11·2	0·2
86	11·05	11·2	0·15
89	11·0	11·25	0·25
98	11·0	11·2	0·2

servations. They found that prolonged experiments lower the optimum temperature of the enzyme.

The effect of hydrogen-ion concentration on the optimum temperature of the amylolytic enzyme also was studied. The digests were set up at p^H 6.4, 7.8 and 8.4 using mixtures as shown in Table VIII. The temperatures at which they were set up were the same as for the previous set of experiments. It was observed that there was no appreciable difference in the optimum temperature when the hydrogen-ion concentration is changed (Fig. 15).

TABLE VII

INFLUENCE OF TIME ON TEMPERATURE OPTIMUM

10 ml of extract and 10 ml. of 2% starch solution at various temperatures
p^H of experiment, 7·2
Control with boiled extract
Duration of experiment, 8, 16, 24 & 32 hrs.

Temperature °C	Experiment vol. in ml. required for 5 ml. Benedict's solution	Control vol. in ml. required for 5 ml. Benedict's solution	Difference in ml.
Duration of experiment 8 hrs.			
40	6·7	12·1	5·4
43	5·7	12·0	6·3
46	5·4	12·2	6·8
49	5 2	12 25	7·05
52	4·7	12·2	7·5
55	4·8	12·05	7·25
58	6·1	12·1	6·0
61	7·0	12·1	5·1
Duration of experiment 16 hrs.			
40	6·9	11·95	5·05
43	6·1	12·0	5·9
46	5·1	12·2	7·1
49	4 8	12 2	7·4
52	5·0	12·3	7 3
55	5·2	12·2	7·0
58	5·5	12.2	6·7
61	6·5	12 2	5·7

Temperature °C	Experiment vol. in ml required for 5 ml. Benedict's solution	Control vol. in ml. required for 5 ml Benedict's solution	Difference in ml.
Duration of experiment 24 hrs.			
40	6·5	12·4	5·9
43	6·1	12·5	6·4
46	5·3	12·55	7·25
49	4·8	12·4	7·6
52	4 7	12·4	7·7
55	5·9	12·4	6·6
58	6·4	12·4	6·0
61	7·1	12·5	5·4
Duration of experiment 32 hrs.			
40	5·9	12·2	6·3
43	5·3	12·2	6·9
46	4·8	12·4	7·6
49	4·4	12.35	7·95
52	4·05	12·2	8·15
55	4·8	12·25	7·45
58	5·25	12·35	7·1
61	5·6	12·3	6·7

TABLE VIII

INFLUENCE OF p^H ON TEMPERATURE OPTIMUM

10 ml. of extract and 10 ml. of 2% starch solution at various temperatures
Duration of experiment, 4 hrs.
Control with boiled extract
p^H of experiment, 6·4, 7·8 and 8·4

Temperature °C	Experiment vol. in ml. required for 5 ml. Benedict's solution	Control vol. in ml. required for 5 ml. Benedict s solution	Difference in ml.
p^H of experiment, 6·4			
40	7·4	11·05	3·65
43	7·1	11·0	3·9
46	6·6	11·1	4·5
49	6·1	11 1	5·0
52	5·6	11·1	5·5
55	5·4	11 0	5·6
58	6 5	11·1	4·6
61	6 9	11·0	4.1
p^H of experiment, 7·8			
40	7·15	11·2	3·45
45	6·8	11·1	4·3
46	6 5	11·2	4·7
49	6·2	11·15	4·95
52	5·4	11·15	5·75
55	5·5	11·1	5·6
58	5·9	11·2	5·3
61	6·3	11·2	4·9
p^H of experiment, 8·4			
40	8·05	11·15	3·1
43	6·9	11·1	4·2
46	6·6	11·15	4·55
49	6·3	11·1	4·8
52	6·0	11·15	5·15
55	6·25	11·2	4·95
58	6·7	11 1	4·4
61	7·7	11·15	3·45

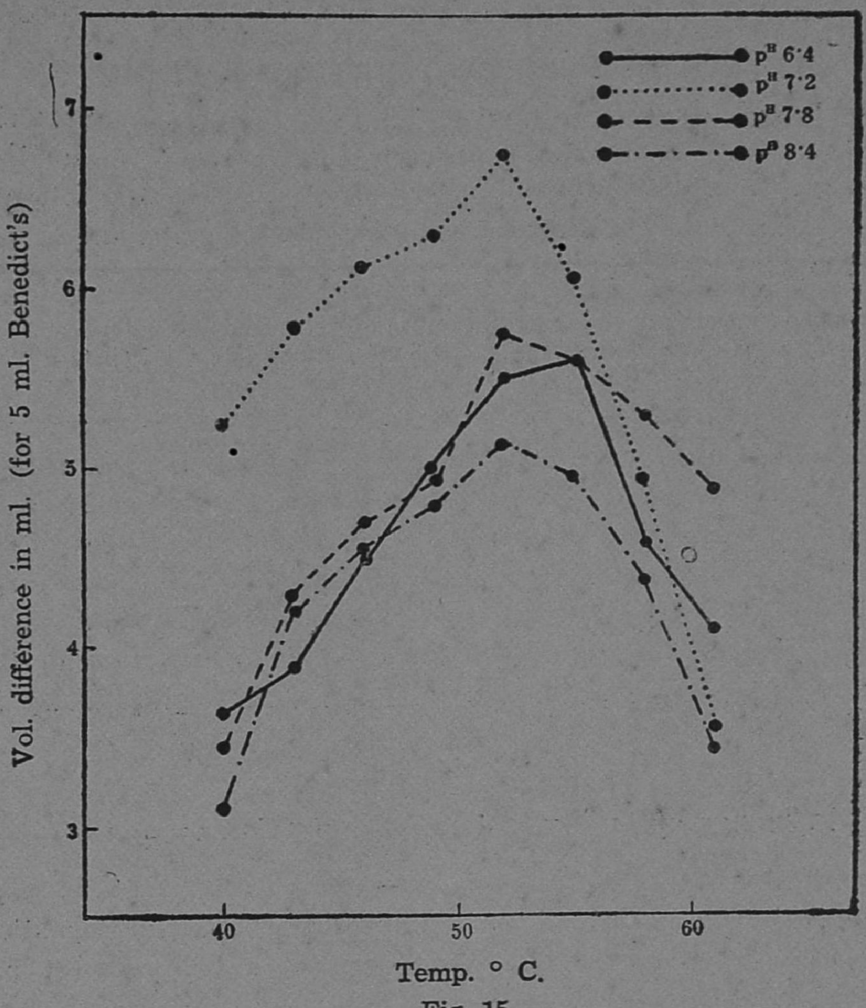


Fig. 15.

Curves illustrating the influence of pH on the temperature optimum.

THE DIGESTION OF FAT

Preliminary experiments revealed that the hepatopancreatic extract of *Penaeus indicus* contains a fat-splitting enzyme. In one set of experiments, the following method of determination of the presence of lipase was adopted. 10 ml. of fresh cow's milk was boiled in a beaker and was made alkaline to Phenol red by adding sufficient quantity of N/20 Sodium carbonate solution. The mixture was then separated into two equal portions in separate containers. To one of these, 5 ml. of 20% hepatopancreatic extract was added and mixed thoroughly. The other was kept as control by adding another 5 ml. boiled extract. Both the mixtures were incubated at 32°C. in a constant temperature apparatus and after twenty hours, the colour in the experimental digestive mixture had changed from red to a definite yellow, while that in the control flask remained red. This observation indicates the presence of a lipase in the extract

and the action noticed might be due to the conversion of butyrin into butyric acid by the enzyme (Yonge, 1924).

The range of action of the lipolytic enzyme is apparently wide. Digests were prepared with olive oil emulsion, lecithin and methyl acetate as shown in Table IX. The results obtained in the experiments show that all the above substances are digested by the extract.

Influence of temperature on the digestion of fat

The temperature of destruction of the lipolytic enzyme was found by heating the extract at different temperatures for fifteen minutes and then incubating with methyl acetate solution as shown in Table X. The digests were set at temperatures of 55, 60, 65, 70, 75 and 80°C. The results obtained show that the lipase is almost completely destroyed between 65 and 70°C., by heating for fifteen minutes. The progressive destruction of the enzyme at higher temperatures is represented graphically in Fig. 16.

Influence of hydrogen-ion concentration on the digestion of fat

The optimum hydrogen-ion concentration for the digestion of fat was studied by setting up identical experiments at pH 6.6, 7.0, 7.2, 7.4, 7.6, 7.8 and 8.2. The volume of NaOH solution required for titration of the fatty acids formed in the experiments as well as the controls are given in Table XI. It will be noticed from the differences in volumes required for titration that the optimum p^H for fat digestion is about 7.4. Fig. 17 shows graphically the influence of p^H changes on the digestive activity of the lipase.

THE DIGESTION OF PROTEINS

The hepatopancreatic extract of *Penaeus indicus* contains a proteolytic enzyme as will be seen from experiments conducted to study the specificity of the enzyme. The results obtained (Table XII) show that the hepatopancreatic extract digests fibrin, peptone, and gelatine. Repeated trials with coagulated egg albumin gave negative results only. Fibrin was found to dissolve completely in the extract and such a digest was incubated for two days and a little acetic acid was used to precipitate undigested globulin. After filteration the following tests (Roaf,

TABLE IX

ACTION OF THE ENZYME EXTRACT ON FATS

Temperature of experiment, 32°C
Duration of experiment, 48 hrs.
p^H of experiment, 7·6
Control with boiled extract

Digestive mixture	Experiment vol. of N/50 NaOH in ml. required for titration	Control vol. of N/50 NaOH in ml. required for titration
10 ml. extract and 2 ml. 1% methly acetate	(i) 3·75 (ii) 3·9	2·3 2·4
10 ml. extract and 2 ml. 2% lecithin solution	(i) 0·55 (ii) 0·60	0·35 0·3
10 ml. extract and 2 ml. olive oil emulsion	(i) 2·7 (ii) 3·1	1·2 1·3

TABLE X

TEMPERATURE OF DESTRUCTION OF LIPASE

10 ml. of extract heated for 15 minutes at various temperatures and 2 ml. 1% methyl acetate
Duration of experiment, 48 hrs.
p^H of experiment, 7·6
Incubated at 32°C

Temperature °C	vol. of N/50 NaOH in ml. required for titration
55	3·6
60	3·1
65	2·6
70	2·2
75	2·1
80	2·1

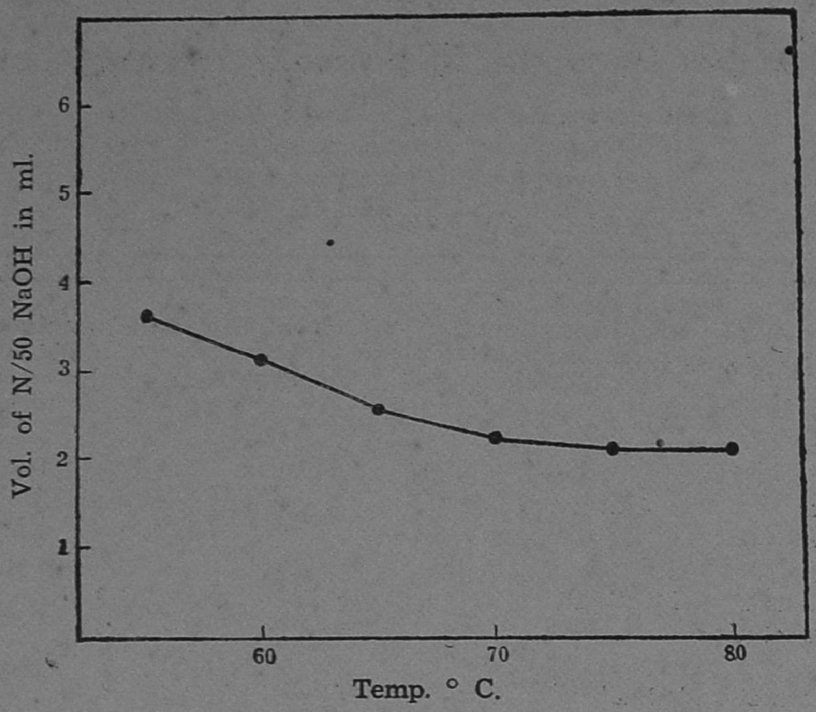


Fig. 16.

Curve illustrating the progressive destruction of the lipase at various temperatures after 15 minutes' heating.

1906; Yonge, 1924) were applied. In biuret reaction (Bodansky and Fay, 1947), a very weak violet colour was produced showing a feeble reaction. Millon's test (Morrow, 1935) showed a moderate red colour and addition of Bromine water showed a pink colouration. These tests reveal the presence of amino acids, but negligible amount of albuminoses only are indicated. Similar results have been obtained by Yonge (1924) for *Nephrops.*

Influence of temperature on the digestion of gelatine

The temperature of destruction of the enzyme was determined by heating the extract to various temperatures for fifteen minutes and then incubating with gelatine at 32°C. The results obtained by titration are presented in Table XIII and Fig. 18, and it will be observed that the enzyme is destroyed by heating the extract for fifteen minutes at about 65°C.

Influence of hydrogen-ion concentration on the digestion of gelatine

Influence of p^H on the digestion of gelatine was found out by the method of observing the liquefaction of gelatine. The procedure is indicated in table XIV and the results show that

TABLE XI

INFLUENCE OF p^H ON THE DIGESTION OF METHYL ACETATE

10 ml. extract and 2 ml. 1% methyl acetate solution
Duration of experiment, 48 hrs.
Temperature of experiment, 32°C
Control with boiled extract

Average p^H	Experiment vol. of N/50 NaOH in ml. required for titration	Control vol. of N/50 NaOH in ml. required for titration	Difference in ml.
6·6	2·85	2·20	0·65
7·0	3·2	2·4	0·80
7·2	3·45	2·35	1·10
7·4	3·80	2·3	1·50
7·6	3·85	2·6	1·25
7·8	4·05	2·9	1·15
8·2	3·6	2·85	0·75

TABLE XII

ACTION OF THE ENZYME EXTRACT ON PROTEINS

Temperature of experiment, 32°C
Duration of experiment, 48 hrs.
p^H of experiment, 7·4
Control with boiled extract

Digestive mixture	Experiment vol. of N/20 NaOH in ml. required for titration	Control vol. of N/20 NaOH in ml. required for titration
10 ml. extract and 10 ml. 2% gelatine	(i) 7·4	3·2
	(ii) 7·2	3·05
10 ml. extract and 0·5 gm. fibrin	(i) 9·4	3·7
	(ii) 9·1	3·7
10 ml. extract and 10 ml. 2% peptone	(i) 11·5	4·5
	(ii) 11·3	4·7

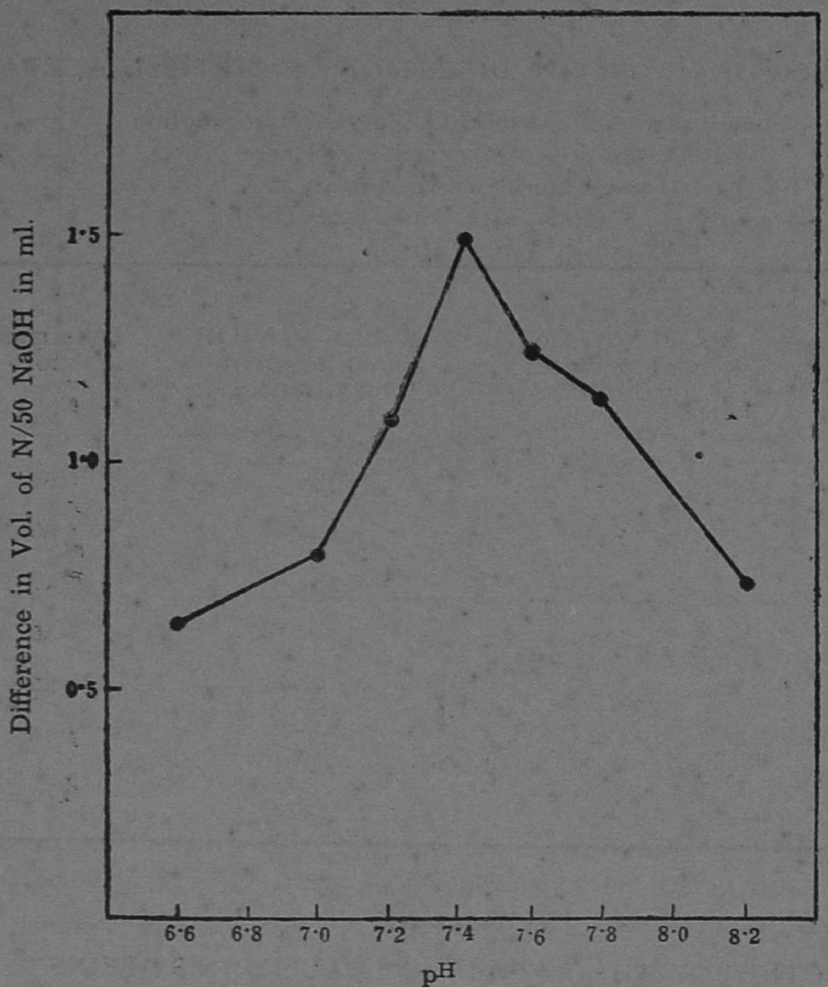


Fig. 17.

Curve illustrating the influence of p^H on the digestion of methyl acetate.

the enzyme is active at p^H 6.8 and continues to be so up to p^H 8.0. Experiments to determine the p^H optimum, using the Sorensen's formal-titration method did not give satisfactory results.

Preliminary experiments were conducted to find out the action, if any, of the extract towards amygdalin, salicin and phloridzin. In all instances the results were negative.

Absorption

To find out the range of absorption of the digested food materials in *Penaeus indicus,* previously starved prawns were taken and a sample of olive oil emulsion (George, 1952) stained with Sudan III was introduced into the cardiac stomach by means of injection syringe. After periods of twentyfour and fortyeight hours, the tissues were dissected out and examined under the microscope for the presence of oil globules. Minute

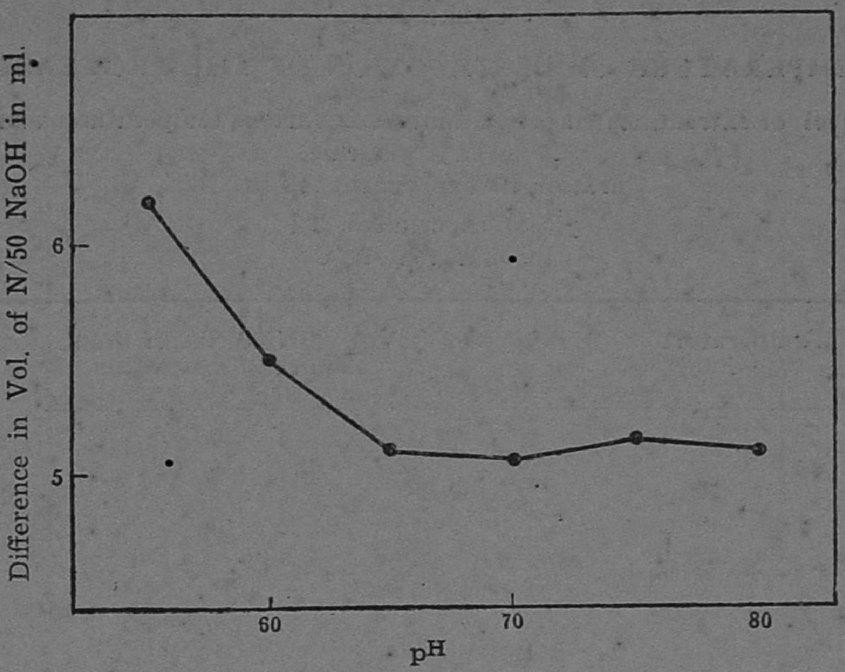


Fig. 18.

Curve illustrating the progressive destruction of the protease at various temperatures after 15 minutes' heating.

red globules could be located only in the mid-gut epithelium and not even traces were found in the epithelial cells of the fore- and hind-gut regions. In the case of the hepatopancreas the olive oil method is not useful because of the presence of stored fat. Frozen sections of the gland from fresh specimens showed the presence of fat in the absorptive cells. However, some specimens were starved for about thirty days in the laboratory tanks and then the stained oil emulsion was introduced into the stomach. After fortyeight hours, the hepatopancreas was dissected and sectioned. When the frozen sections were examined under the microscope, very small globules were seen in some of the cells of the tubules, but this evidence is not conclusive. Hence specimens were fed on ferrous lactate solution also (Yonge, 1924) to find out the absorptive nature of the cells of the hepatopancreas. The glands of these animals fed on ferrous lactate solution were fixed in ammonium sulphide in 95% alcohol (Reddy, 1937) for sectioning. The sections showed the presence of very small amounts of the iron salt in the epithelial region of the tubules.

Some specimens were fed on methylene blue and after twenty hours, the alimentary canal was dissected out and sectioned. The absorbing nature of the epithelial cells of the mid-gut could be detected by the presence of the stain granules in

TABLE XIII

TEMPERATURE OF DESTRUCTION OF THE PROTEASE

10 ml. of extract heated for 15 minutes at various temperatures and
10 ml. 2% gelatine
Duration of experiment, 48 hrs.
p^H of experiment, 7·4
Incubated at 32°C

Temperature °C	Vol. of N/20 NaOH in ml. required for titration
55	6 2
60	5·5
65	5·1
70	5 05
75	5·15
80	5·1

TABLE XIV

INFLUENCE OF p^H ON THE DIGESTION OF GELATINE AS INDICATED BY LIQUEFACTION

10 ml. 2% gelatine in the buffer solution and 5 ml. extract incubated
for 48 hours at 32°C and placed in ice for 30 minutes
Control with boiled extract

Average p^H	Condition after placing in ice	Condition of control
6·2	Solid	Solid
6·4	Solid	Solid
6·8	Semi-fluid	Solid
7·0	Fluid	Solid
7·4	Fluid	Solid
7·8	Fluid	Solid
8·0	Semi-fluid	Solid

the cytoplasm, around the nuclei. In the hepatopancreas, lightly coloured granules could be seen in many cells. Yonge (1924) states that the food which is easily soluble is passed immediately from the gut to the hepatopancreas and only less soluble substances such as fat pass into the mid-gut.

Discussion

The anatomical and histological features of the alimentary canal of *Penaeus indicus* resemble in general those of *Palaemon* (Patwardhan, 1937). The structural differences which have been noted are probably due to the food and feeding habits of the animal. Such for example is the comparative reduction of the gastric mill. The relation between the food and reduced gastric mill has already been pointed out by Patwardhan (1935). Considerable variations have also been observed in the relative length of the mid-gut in different decapods. In many of the forms that have been studied, the mid-gut is the shortest portion (Frenzel, 1885; Pearson, 1908; Reddy, 1937). In *Penaeus indicus*, the mid-gut is the longest portion of the alimentary canal. Similar observations have been made on *Nephrops*, and *Neptunus* (Wallengren, 1901, Yonge, 1924, and George, 1949.).

The tegumental glands have been observed in relation to the fore-gut and hind-gut of decapods by a number of workers (Wallengren, 1901; Pearson, 1908; Jackson, 1913; Farkas, 1914; Yonge, 1924, 1932). A variety of functions have been attributed to the tegumental glands of decapods (See Yonge, 1932), as for example excretion in *Astacus* (Lang, 1891), sensory function in *Homarus* (Herrick, 1896) and slime secreting function in several forms. Many authors following Braun (1877) described them as salivary glands, but no experimental evidence has so far been put forward. In view of the fact that no digestive powers could be demonstrated with extracts of the oesophagus of *Penaeus indicus*, it appears likely that these glands do not have a digestive function in the prawn. On the other hand, evidence has been brought forward to suggest that the oesophageal glands of decapods may be related to the secretion and preservation of the chitinous lining (Yonge, 1924, 1932, 1936; Richards, 1951). Yonge (1932) observes that due to the presence of the digestive enzymes in the hepatopancreatic secretion, a salivary secretion in the oesophagus is not necessary for the decapods.

From a study of the properties of the hepatopancreatic extract of *Penaeus indicus,* it appears that carbohydrates, proteins and fats are all effectively digested. The existence of separate enzymes in the digestive glands has been shown in many crustaceans, and it is supposed that the nature and strength of the digestive enzymes are correlated to the feeding habits of the animal concerned (Yonge, 1931, 1937; Vonk, 1937). The hepatopancreatic extract of *Penaeus* is able to digest all the three types of food substances and this is related to the omnivorous habit of the animal (Gopalakrishnan, 1952). Other groups of omnivorous animals such as annelids and some echinoderms also possess such enzymes (Yonge, 1937). Carnivorous forms like coelenterates, asteroids, stomatopods and cephalopods have strong proteolytic enzymes only (Petrievic, 1915; Yonge, 1930, 1937). An exception to this rule is found in the carnivorous form *Peripatopsis* (Heatley, 1936), in which the proteolytic enzyme is weaker compared to the amylase. In the lamellibranchs, herbivorous gastropods, cirripedes and tunicates, the proteolytic enzymes are very weak (Yonge, 1923, 1937; Berril, 1929; Graham, 1931 a). The studies on the physiology of digestion in insects have shown the existence of relationship between the nature of the food and digestive enzymes (Wigglesworth, 1934; Yonge, 1937).

It is clear from the study of the digestion of carbohydrates in *Penaeus indicus* that the hepatopancreatic secretion is able to act on a variety of carbohydrates such as starch, sucrose, glycogen, maltose and lactose. While this seems to be the general rule in the decapods so far studied, the range of activity and the variety of substances digested by the enzyme may be variable, as is seen from the observations by Roaf (1906, 1908 b), Yonge (1924), Kruger and Graetz (1928), Wiersma and Van der Veen (1928) and Reddy (1937). It is known that invertebrates do not show the same degree of specificity of enzyme action as vertebrates, a single enzyme being effective on a variety of substances (Yonge, 1937; Vonk, 1937).

The hepatopancreatic extract of *Penaeus indicus* has no effect on inulin, raffinose, cellulose (saw dust) and pentosans (gum arabic). Considerable amount of carbohydrate is present in the detrius as cellulose and pentosans (Boysen-Jensen, 1914 quoted by Nicol, 1931). Hence the absence of any reaction of the extract towards saw dust and gum arabic is interesting in view of the suggestion made that the prawns eat detritus also (Gopalakrishnan, 1952). Enzymes acting on these sub-

stances have not been met with in the alimentary canal of any of the detritus feeding forms among decapods, echinoderms, lamellibranchs, polychaetes and tunicates (Nicol, 1931), and hence it has been suggested that detritus may not be a source of nourishment to these animals. Evidences for the presence of cellulose have been obtained in the boring molluscs *Teredo* and *Bankia* and also a number of insects (See Yonge, 1938). It has been suggested that the cellulose reported in *Astacus* is hemicellulose (Yonge, loc. cit.).

In the foregoing study an attempt has been made to find out the influence, if any, of the hydrogen-ion concentration and temperature on enzyme action. It has been shown in different animals that the action of the digestive enzyme is dependent on the hydrogen-ion concentration of the substrate (Roaf, 1906; Yonge, 1931, 1937; Vonk, 1937). The activity-p^H curve for the action of the amylolytic enzyme of *Penaeus* shows that the digestive activity is at its maximum in a neutral medium. (In some experiments the optimum lay between p^H 7.0 and 7.2). Experiments were also conducted to find out the effect of changes in temperature and duration of the experiment on the optimum p^H of the enzyme action. It was observed that both the factors do not affect the p^H optimum. Graham (1931 b) has shown that in *Pecten maximus* the p^H optimum is not affected by variations in time of the experiment. The optimum temperature also for the amylase of *Penaeus* is not affected by changes in duration of the experiment and hydrogen-ion concentration of the substrate. Similarly, in *Sabella* the optimum temperature for starch digestion is not affected by changes in duration of the experiment and p^H (Nicol, 1931). On the other hand, Berril (1929) found that in the ascidian *Tethyum*, experiments of brief duration indicate an optimum temperature for enzyme action at about 40°C., while more prolonged ones indicate a much lower value, less than 20°C., after about fortyfive hours. The studies of Graham (1931 b) on *Pecten* showed that a direct relationship exists between the hydrogen-ion concentration and the optimum temperature for enzyme action, and this relationship may be compared to findings of Compton (1915) for the enzymes of yeast.

The fat-splitting enzymes of invertebrates have been shown to be different in their composition from those of vertebrates (Vonk, 1937). The lipolytic enzyme of *Penaeus indicus* appears to show a wide range of activity as may be inferred from its digestive action on lecithin, olive oil, and methyl acetate.

Similar widespread activity has been noted by Yonge (1924) in *Nephrops*, and Reddy (1937) in *Paratelphusa*. Yonge found that the hepatopancreatic extract of *Nephrops* digests olive oil, methyl acetate and ethyl butyrate. The lipolytic enzyme of *Paratelphusa* hybrolyses methyl acetate, amyl acetate, butyl acetate, ethyl acetate and an emulsion of olive oil. Vonk (1935) observed the emulsification of fat by the secretion of *Astacus*. The enzyme of *Penaeus indicus* appears to have a better action on fat than on esters, as was observed from the experiments conducted. Whether such differential activity indicates a qualitative difference to the enzyme concerned is not certain. Kruger and Graetz (1927 b) state that the lipolytic enzyme of *Astacus* is more like an esterase. On the other hand, Kruger (1929) showed that the enzyme in *Homarus* and *Palinurus* appears to be more like a lipase. Vonk (1927) explains that in the same species of an animal, sometimes the action on fat and at other times the action on esters is stronger than the other.

The presence of a proteolytic enzyme capable of digesting fibrin, peptone and gelatine in the hepatopancreatic extract is also evident from the foregoing study. In *Carcinus* (Roaf, 1906), *Nephrops* (Yonge, 1924) and *Paratelphusa* (Reddy, 1937), the maximum activity of the enzyme is in a medium of N/20 Na_2CO_3. In *Penaeus* the enzyme is active between p^H 6.8 and 8.0. It has been found possible to isolate four components of the proteolytic enzyme from the mid-gut gland of the crab *Maja squinado* (Mansour-Bek, 1932). It is also known that the digestive juice of *Astacus* contains a protease and polypeptidase and three hydrogen-ion optima have been described for the action of the juice on different substances (Shinoda, 1928). Widely different results have been obtained by Kruger and Graetz (1927 a, 1928) for the proteases of the same animal. As observed by Vonk (1937) the differences in the findings of Shinoda, and Kruger and Graetz are not easily explainable. The conclusion of Kruger (1929) that the digestive juice of *Astacus* contains four different enzymes has not yet been proved and in this connection Vonk (1937) states that the various actions noted may be due to a single enzyme with special properties, not described in other instances.

SUMMARY

1. The anatomical and histological features of the different parts of the alimentary canal of *Penaeus indicus* are described.

2. The passage of food materials through the different regions of the alimentary canal is studied by experimental feeding.

3. The hepatopancreas is shown to be responsible for the production of the various digestive enzymes.

4. Experimentally it has been observed that different carbohydrates such as starch, sucrose, maltose, lactose and glycogen are digested by the hepatopancreatic extract, but not inulin, cellulose, raffinose and pentosans. The optimum hydrogen-ion concentration for digestion of starch is between 7.0 and 7.2. This optimum is not affected by changing the period of digestion and also the temperature of the experiment. The optimum temperature for the action of the enzyme is about 52°C., and the temperature of destruction between 71° and 74° and the optimum is not affected by changes in period of experiment or changes in hydrogen-ion concentration.

5. Lipolytic enzyme of the prawn digests lecithin, methyl acetate and olive oil. The enzyme gets destroyed between 60° and 65°C. The optimum p^H for the digestion of fat is 7.4.

6. The proteolytic enzyme digests fibrin, peptones and gelatine, the action of the enzyme being maximum in an alkaline medium. The temperature of destruction is about 65°C.

7. Absorption of digested food materials appears to take place in the mid-gut region of the alimentary canal and also the absorptive cells of the hepatopancreatic tubules.

8. The general problems concerning the alimentary canal and physiology of digestion in the prawn are discussed.

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PART II

THE OXYGEN CONSUMPTION OF *METAPENAEUS MONOCEROS* IN RELATION TO ENVIRONMENTAL CONDITIONS

II. THE OXYGEN CONSUMPTION OF *METAPENAEUS MONOCEROS* IN RELATION TO ENVIRONMENTAL CONDITIONS.

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THE OXYGEN CONSUMPTION OF *METAPENAEUS MONOCEROS* IN RELATION TO ENVIRONMENTAL CONDITIONS

INTRODUCTION

It has been pointed out that in a number of euryhaline invertebrates the rate of oxygen consumption varies inversely to the changes in salinity of the external medium (See Schlieper, 1930; Lowenstein, 1935; Krogh, 1939; Hopkins, 1949). The oxygen consumption of crustacean types in relation to changes in salinity of the medium has attracted the attention of earlier workers (vide supra). Kreps (1929) showed that the rate of oxygen intake of *Balanus crenatus* is not affected by changes in salinity between 12 °/₀₀ and 30 °/₀₀ and that below a level of 12 °/₀₀ the rate decreases considerably. Schlieper (1931) observed that the respiratory rate of *Gammarus locusta* is lower in sea water than in brackish water and that in different concentrations of brackish water higher rates are noticed only in very dilute solutions. The studies of Fox and Simmonds (1933) revealed that in related species of amphipods and isopods the rate of oxygen consumption is higher in freshwater types than in marine ones. The researches of Schwabe (1933) proved that in hypotonic media the rate of oxygen consumption of *Carcinus maenas* increases considerably. Marshall, Nicholls and Orr (1935) studied the oxygen consumption of the copepod *Calanus finmarchicus* in relation to salinity changes of the medium and their results indicated that whereas in media of salinities 33.91 °/₀₀ and 27.16 °/₀₀ the rate is practically constant, in a medium containing 50% sea water (Salinity 17 °/₀₀) the rate is about 35% lower. It appears reasonable to infer from the observations recorded so far, that osmotic behaviour of animals has a bearing on the rate of oxygen consumption. In a recent investigation Panikkar and Viswanathan (1948) showed that *Metapenaeus monoceros* is able to adapt itself to waters of varying salinity, and presumably such osmoregulatory powers are shown by other migratory penaeids also (Menon, 1952). In the light of the relationship existing between osmoregulatory activity and the rate of oxygen consumption, it is of interest to study the respiratory behaviour of these animals in media of different salinities. Further, the oxygen consumption of tropical aquatic animals has not received

much attention and so the present work on oxygen consumption of the brackish water prawn *Metapenaeus monoceros* was thought desirable.

The rate of oxygen consumption of animals has also been shown to vary with changes in temperature. After Krogh's (1916) survey of the studies on the influence of temperature on the oxygen consumption of different animals, this aspect of respiration in crustaceans was further investigated by Marshall, Nicholls and Orr (1935), Fox (1936), Fox and Wingfield (1937), Clarke and Bonnett (1939), Edwards and Irving (1943) and Edwards (1946). In the copepod *Calanus finmarchicus* it was observed that an increase of temperature from 0°C. to 20°C. accelerates the respiratory rate (Marshal *et al*, loc. cit.). That the temperature of the external medium affects the rate of oxygen consumption is also shown by Fox (1936) who found that closely related species of *Pandalus* living in arctic waters and in English seas show different respiratory rates, those in the warmer waters showing a greater rate of oxygen consumption. Incidentally, it has also been shown that the curves relating oxygen consumption to temperature for *Pandalus borealis* from arctic waters and *Pandalus montagui* from Plymouth are parallel to the corresponding curves for the isolated muscles of the animals, thereby suggesting that the greater oxygen consumption of animals in warmer waters may be due to a greater non-locomotary metabolism (Fox and Wingfield, 1937). Capraro (1939) studied the rate of oxygen consumption of *Carcinus maenas* at different temperatures and the results suggest that the rate is directly proportional to the temperature changes of the medium. In *Emerita talpoida* it has been observed that the oxygen consumption increases with temperature, but is brought back to normal when the original temperature is restored (Edwards and Irving, 1943). The above authors found that the rate of oxygen consumption of winter and summer *Emerita talpoida* increases with rises in temperature and that the maximum oxygen consumption of the animals in winter is at 23°C., whereas that of animals in summer is at a slightly higher temperature of about 30°C. It will be noted that all the above studies were on non-tropical types adapted to sharply marked seasons and wide temperature ranges. Since in the tropics there is no clear differentiation of the seasons, the temperature ranges are narrow, between about 25° and 33.8°C., (Panikkar and Aiyar, 1937) and the average temperature is higher, it was felt that the relationship between temperature and oxygen consumption might not be so direct as in those living in colder

regions. Further it seemed possible, as has been suggested by Irving * that the arctic and tropical crustaceans may show similar correlation between oxygen consumption and temperature, but the tropical ones acclimatized to higher temperature conditions may show marked decrease in rate of oxygen consumption at lower temperature ranges. Very recently Scholander *et al* (1953) have studied the oxygen consumption of a number of arctic and tropical cold-blooded animals at different temperatures and found that there is considerable metabolic adaptation in the arctic forms compared to the tropical forms.

The rate of oxygen consumption of animals, in addition to being susceptible to changes in salinity and temperature of the external medium is known also to react to a gradient of acidity and alkalinity, and it has been shown (Powers, 1930) that some forms react directly to a definite range of hydrogen-ion concentration of the sea water while in others the reaction is less definite and still others seemingly do not respond to differences in alkalinity or acidity. Detailed studies on this aspect have been made in many fishes (Powers, 1922, 1930, 1932) but so far invertebrates, especially Crustacea, are concerned there are very few studies recording the effect of hydrogen-ion concentration of the external medium on the rate of oxygen consumption. Helff (1928) found that changes in p^H have no effect on the oxygen consumption of the crayfish *Cambarus immunis*. Marshall *et al* (1935) have studied the effect of p^H changes on the respiration of *Calanus finmarchicus* and observed that the effect of p^H changes on the rate of oxygen consumption is negligible within the limits studied, i.e., between p^H 8.08 and 7.40. In view of the paucity of information on the relation, if any, between oxygen consumption of tropical crustaceans and p^H of the medium, experiments were conducted to study the rate of oxygen consumption of prawn at different levels of p^H. It may also be mentioned that changes in hydrogen-ion concentration in sea water are of importance to marine animals, eventhough the differences are slight compared to those in fresh water (Power, 1930; Marshall *et al*, 1935).

The relationship between dissolved oxygen concentration of the external medium and the rate of oxygen consumption in many aquatic invertebrates has been studied by earlier authors and it is known that this relationship is variable (See Krogh, 1916; Helff, 1928; Hyman, 1929; Hiestand, 1931; Marshall *et al*,

* Personal Communication.

1935). It has also been shown that such variations occur in closely related animals, as for example different species of ephemerid nymphs (Fox, Wingfield and Simmonds, 1937). In many of the animals that have been studied, the results show that while an increase in oxygen content is not likely to harm the animals, reduction of the same to very low levels produces harmful effects. However, in some animals there has been observed a direct and uniform relationship between dissolved oxygen content and rate of oxygen consumption (See Hyman, 1929; Hiestand, 1931). In view of the fact that the relationship between the two factors vary considerably, it was thought desirable to study the influence of changes in dissolved oxygen content also on the rate of oxygen consumption of *Metapenaeus monoceros.*

Material and Methods

The prawns used for this study were collected from the mouth of the Cooum river, which flows into sea during the monsoon period when the freshets swell the river and at other times the river mouth is blocked by a sand bar thrown by the breakers. Hence the salinity of the water in this part of the river varies during the latter period. It was noted that when the present study was undertaken the salinity ranged between 20.4 and 21.2 °/₀₀ for a period of thirty days, at the region from which the specimens were collected. The sides of the river are muddy and the bottom is often contaminated by sweage effluents. The amount of dissolved oxygen did not show marked variations, the average being 3.63 ml/L.

The prawns used in this investigation were stocked in the laboratory aquaria in sea water, the salinity of which was reduced to 20 °/₀₀ by addition of the required quantities of fresh water. The prawns were fed with algae, fish and lamellibranchs. To prevent pollution of water by faecal matter, the prawns were starved for one full day before using for the experiments, as was done in the case of fishes by Basu (1949). Medium sized prawns (females only) were chosen for the experiments. The quantity of oxygen consumed was calculated in relation to unit dry weight of the prawns and the values thus obtained have been expressed as rate of oxygen consumption in c.mm. oxygen at N.T.P. per gm. dry weight per hour.

A constant flow apparatus, suitable for small quantities of water was set up as shown in Fig. 1. By this means water was

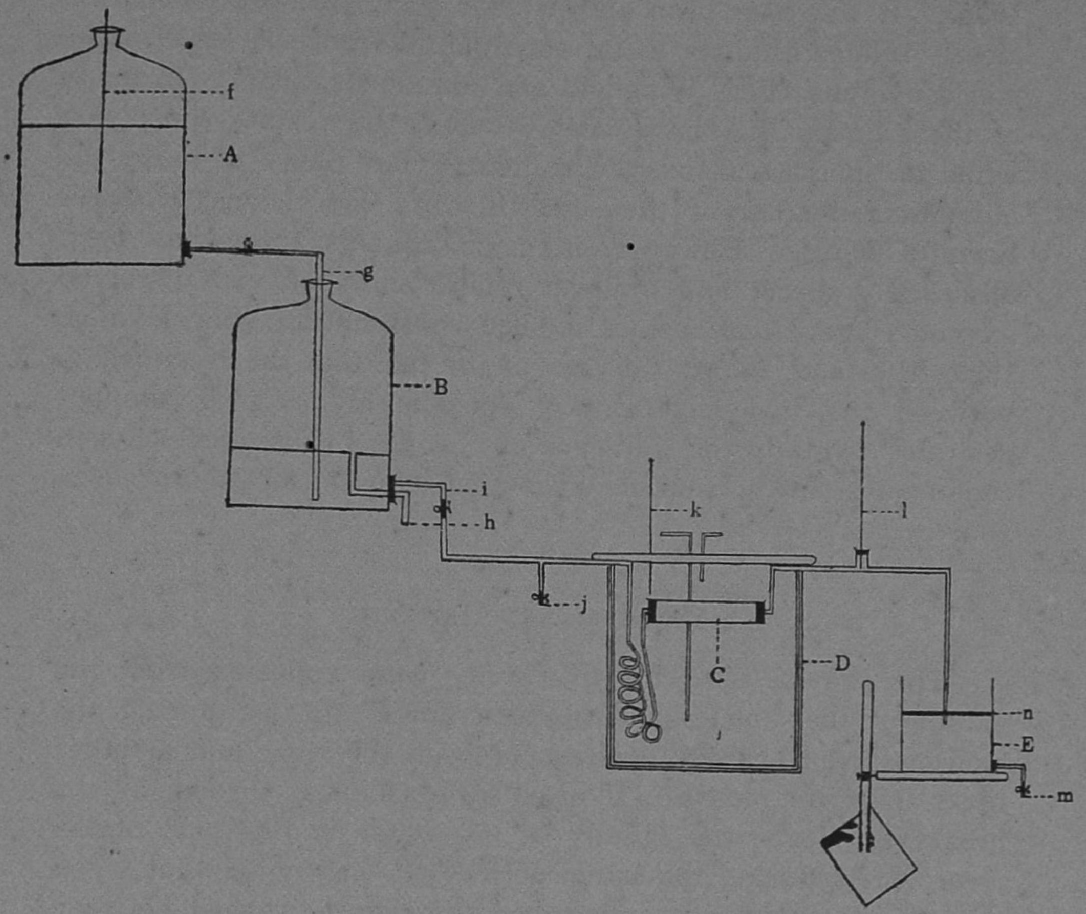

Fig. 1. General scheme of apparatus.

A, B, aspirators
C, respiration chamber
D, bath
E, receiver jar
f, k, l, thermometers
h, overflow tube
g, i, supply tubes
j, m, taps
n, layer of liquid paraffin.

circulated at a uniform rate through the respiration chamber containing the test animal. Tube 'h' (Fig. 1) prevents the water in the second aspirator ('B') from rising above a certain level and as the first aspirator ('A') is at a higher level than 'B' and as the tube 'g' leading water from 'A' to 'B' is stouter than 'i', the water level in aspirator 'B' is kept steady. This ensures a uniform speed for the flow of water through the respiration chamber as all the parts of the apparatus are clamped to fixed positions. To ensure the constant flow of water it is

also necessary to keep the negative pressure at the free end of the tube delivering the water from the respiration chamber constant. At the outset this end of the tube dips into a two cm. thick layer of liquid paraffin poured into the receiver. When the experiment is started, water collects in the vessel under this spread of oil and direct contact with air is prevented. But as more water accumulates in the receiver, the increased negative pressure reduces the rate of flow and to avoid this the depth to which the free end of the tube dips into the water is adjusted, by lowering the receiver suitably (Keys, 1930).

The rate of flow of water was calculated by measuring the volume of water collected in the receiver during a period of one hour. The oxygen content of the water flowing into the respiration chamber was estimated from samples tapped out from the tube 'j' while prawns were introduced and also at the close of each half hour. On each occasion two identical samples were drawn out and duplicate determinations were always made. The oxygen content of the outflow was determined by filling two sampling bottles through the tube 'm' leading from the receiver jar and then making duplicate determinations. In filling these sampling bottles care was taken to allow the water to overflow for two minutes so as to remove the upper layers of water in contact with air already present in the bottle. As it was necessary to run the experiment without interruption for four hours and also to analyse two samples after each hour, the receiver jar 'E' was taken away at the end of the hour and an identical one was substituted. While this was getting filled, the samples from the first hour outflow water could be analysed. As the oxygen content of the inflow water as well as the outflow water varies with temperature, thermometers 'f' and 'l' were frequently read. In order that the temperature of the flowing water in the respiration chamber ('C') is maintained constant for a fairly long period, a wide-mouthed thermos-flask was used as the bath 'D'. Necessary addition of cold or warm water kept the temperature of the water in the bath within a desired range. The temperature of the water in the respiration chamber is measured through a 'T' tube inserted into its outflow. With practice it was found possible to regulate the temperature of the flowing water within a desired range. The bath was kept covered to prevent light interfering (Marshall *et al*, 1935). The size of the respiratory chamber ('C') is such that the test animals kept in it just fit into it and only a minimum amount of movement is possible.

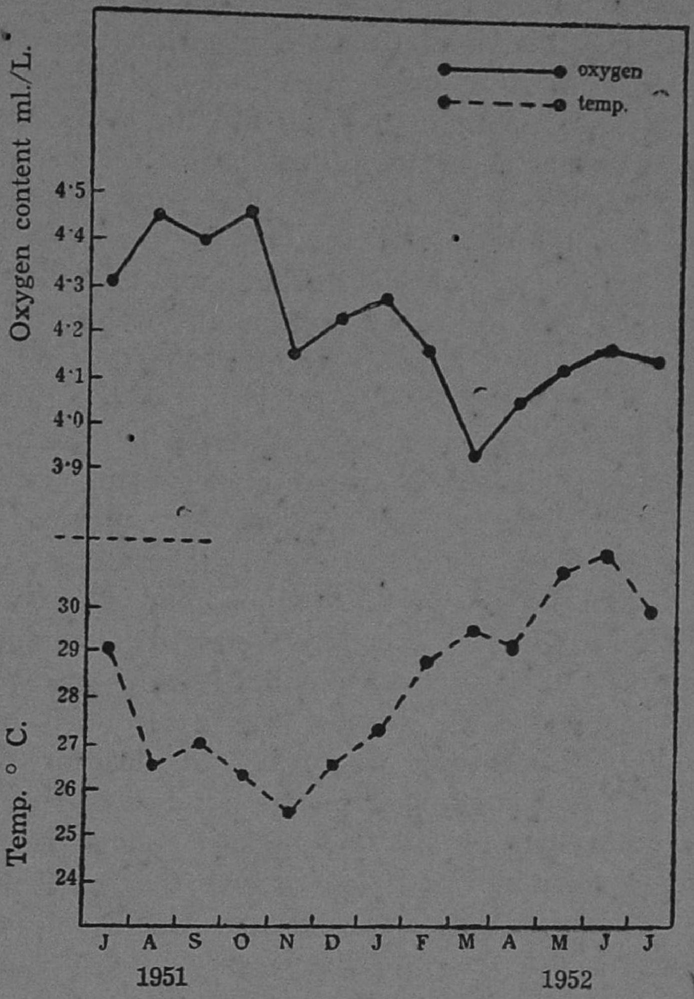

Fig. 2.

Curves illustrating the oxygen content and temperature fluctuations in the Cooum river.

K8634

For determination of the dry weight, the specimens were dropped in boiling water and the body cut into small bits before drying at 105 °C. in a constant temperature oven till constancy of weight was attained (Lowenstein, 1935). The time necessary to get the material thoroughly dried varied between eight and twelve hours.

Salinity estimations were made by titrating with silver nitrate solution (Harvey, 1928). p^H determinations of the water samples were made with a Hellige's comparator and estimation of dissolved oxygen, by the modified Winkler's method (Standard methods of water analysis, 1936).

For all experiments, sea water diluted to the required salinity was used. It was filtered and kept exposed for two hours prior to use, so that the dissolved oxygen content might not

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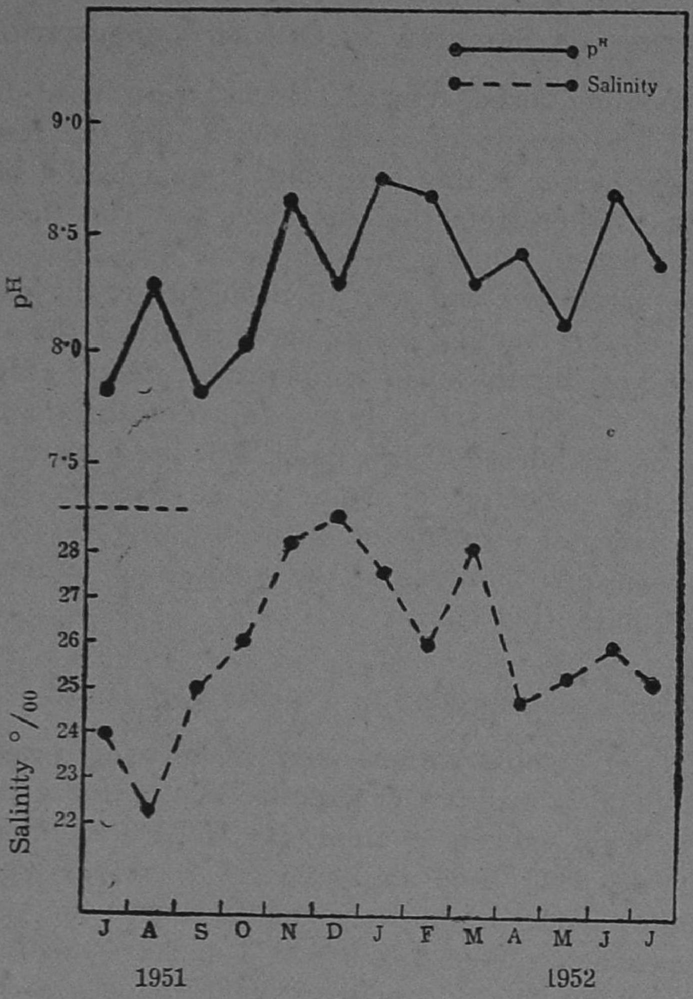

Fig. 3.

Curves illustrating the fluctuations in the salinity and pH of the Cooum river.

change during the four hours' study. Preliminary blank experiments showed that no change in oxygen content occurred when the water was allowed to flow through the apparatus.

The p^H of the water was lowered by passing carbon dioxide gas into the water and it was increased by adding dilute sodium hydroxide solution (Marshall *et al,* 1935). The oxygen tension of the water was increased by oxygenating and lowered by passing successively washed hydrogen (Marshall *et al,* loc.cit.).

The salinity, temperature, hydrogen-ion concentration and oxygen content of the natural habitat of the prawns, viz., Cooum river, were estimated for a period of one year (July 1951—July 1952). The results obtained are presented in Fig. 2 and 3. The water samples for these determinations were taken from the eastern side of the Napier Bridge.

EXPERIMENTAL RESULTS

INFLUENCE OF SALINITY ON OXYGEN CONSUMPTION

Three medium sized female prawns were used for each experiment. The specimens were inserted into the respiratory chamber under water taking care not to damage the body and the pleopods. Water from the aspirators was run through the apparatus for fifteen minutes to remove any possible effects of handling the specimens and also to bring about the change in temperature of the running water, as a result of the water in the bath being of slightly lower temperature. After reading the thermometers 'f' and 'l' (Fig. 1) and adjusting the temperature of the bath, as has already been described, the experiment was started with the collection of water in the receiver 'E'. The rate of flow of water through the apparatus was fiftytwo ml./L. All details regarding analyses of the inflow and outflow water are given in tables (vide infra).

Rate of oxygen consumption in a medium of salinity 30 °/₀₀

The first set of observations were made using prawns that had been kept in a medium of salinity 30 °/₀₀ for a period of four hours. The oxygen content (at N.T.P.) of the inflow water was 4.48, 4.40, 4.59 and 4.56 ml./L. respectively. The p^H of the inflow and outflow were the same and the values for the four experiments were 8.5, 8.6, 8.4 and 8.4 ml./L. respectively. It must be pointed out that slight changes in p^H could not be detected. However, it is thought that such changes in the p^H of the water might not interfere with the values for rate of oxygen consumption (Marshall *et al*, 1935). The oxygen content of the outflow water was found out as described earlier and from the observations thus made, the volume of oxygen consumed during one hour, when a certain volume of water flowed past was calculated. The results obtained in the four experiments (Table I) show that the rate of oxygen consumption in the particular medium, varies between 358 and 578 c.mm. per gm. per hour, the average being 471.

It has been observed in different groups of animals that the acclimatization to concentrated sea water takes place after some time only and that the time taken to reach a constant rate varies in different animals and in different media. In the allied prawns *Leander* and *Palaemonetes*, a steady fall in the internal osmotic pressure occurs for about fourteen to twentyfour hours, when the external medium is diluted, but afterwards a constant

TABLE I

OXYGEN CONSUMPTION IN RELATION TO SALINITY

Salinity 30°/oo *Time after 4 hrs.* *Rate of flow : 52 ml./min.*

Exp. No.	No. of prawns used	Total weight (dry) of prawns in gm.	Period of exp.	Inflow: Mean temp °C	Inflow: pH*	Inflow: Mean oxygencontent in ml./L. at N.T.P.	Outflow: Mean temp °C (for each hr.)	Outflow: pH*	Outflow: Mean oxygen content in ml./L. at N.T.P. (for each hr.)	Rate of oxygen consumption in c. mm./gm./hr.
1	3	2·772	4 hrs.	26·9	8·5	4·48	26·2	8·5	4·07	462
							25·4		4·15	372
							24·5		4·03	507
							26·0		3·98	563
2	3	3·243	4 hrs.	25·7	8·6	4·40	24·8	8·6	3·91	472
							25·2		3·80	578
							24·7		3·87	510
							250		3·89	491
3	3	3·792	4 hrs.	27·0	8·4	4·59	26·5	8·4	4·11	395
							25·6		4·04	453
							24·2		3·90	568
							25·1		3·95	527
4	3	4·623	4 hrs.	25·5	8·4	4·56	24·7	8·4	3·99	385
							25·4		3·84	486
							23·8		4·03	358
							25·2		3·95	412

*Approximate value

Mean 471

TABLE II

OXYGEN CONSUMPTION IN RELATION TO SALINITY

Salinity : 30°/oo *Time : after 16 hrs.* *Rate of flow : 52 ml./min.*

Exp. No.	No. of. prawns used	Total weight (dry) of prawns in gm.	Period of exp.	Inflow: Mean temp °C	Inflow: pH.*	Inflow: Mean oxygen content in ml /L. at N.T.P.	Outflow: Mean temp °C (for each hr.)	Outflow: pH.*	Outflow: Mean oxygen content L. N.T.P. (for each hr.)	Rate of oxygen consumption in c. mm./gm./hr.
1	3	2·703	4 hrs.	27·5	8·4	4·42	25·8	8·4	3·93	566
							24·7		3·98	508
							25·		3·94	554
							25 6		3·84	669
2	3	3·456	4 hrs.	28·1	8·5	4·31	26·1	8·5	3·62	623
							26·7		3·57	668
							23·9		3·66	587
							24·4		3·68	569
3	3	4·392	4 hrs.	26·7	8 4	4·27	26·0	8·4	3·55	511
							25·3		3·63	455
							25·0		3·47	568
							24·7		3·39	625
4	3	4·566	4 hrs.	27·8	8·2	4·36	25·7	8·2	3·53	567
							25·3		3·70	451
							23·8		3·49	594
							24·5		3·65	485
									Mean	563

*Approximate value

TABLE III

OXYGEN CONSUMPTION IN RELATION TO SALINITY

Salinity : 30°/oo *Time : after 24 hrs.* *Rate of flow : 52 ml./min.*

Exp. No.	No. of prawns used	Total weight (dry) of prawns in gm.	Period of exp.	* Inflow			Outflow			Rate of oxygen consumption in c. mm./gm./hr.
				Mean temp °C	pH.	Mean oxygen content in ml./L. at N.T.P.	Mean temp °C (for each hr.)	pH.	Mean oxygen content in ml./L. at N.T.P. (for each hr.)	
1	3	2·577	4 hrs.	28·2	8·3	4·53	25·8	8·3	4·02	618
							26·4		3·98	666
							25·0		4·08	545
							26·3		4·00	642
2	3	3·495	4 hrs.	28·8	8·5	4·24	26·7	8·5	3·60	572
							26·1		3·52	643
							25·7		3·48	679
							24·0		3·55	616
3	3	4·620	4 hrs.	26·6	8·2	4·42	25·4	8·2	3·64	527
							24·5		3·68	500
							25·3		3·60	554
							26·0		3·56	581
4	3	4·824	4 hrs.	27·8	8·3	4·39	27·1	8·3	3·48	589
							26·5		3·44	615
							26·1		3·41	634
							25·7		3·47	595
									Mean	599

*Approximate value

TABLE IV

OXYGEN CONSUMPTION IN RELATION TO SALINITY

Salinity : 30°/oo *Time : after 48 hrs.* *Rate of flow : 52 ml /min.*

Exp. No.	No. of prawns used	Total weight (dry) of prawns in gm.	Period of exp.	Inflow: Mean temp °C	Inflow: pH*	Inflow: Mean oxygen content in ml./L. at N.T.P.	Outflow: Mean temp °C (for each hr.)	Outflow: pH*	Outflow: Mean oxygen content in ml./L. at N.T.P. (for each hr.)	Rate of oxygen consumption in c. mm./gm./hr.
1	3	2·871	4 hrs.	26·9	8·5	4·57	26·7	8·5	4·06	554
							26·2		3·99	630
							26·3		4·03	587
							25·5		3·92	706
2	3	3·258	4 hrs.	26·5	8·4	4·52	26·1	8·4	3·95	546
							26·1		3·91	584
							25·4		3·84	651
							24·9		3·92	575
3	3	4·233	4 hrs.	27·3	8·6	4·35	26·6	8·6	3·74	450
							26·4		3·66	509
							25·9		3·69	487
							26·2		3·63	531
4	3	4·485	4 hrs.	26·8	8·4	4·75	25·8	8·4	4·02	508
							24·5		4·00	522
							25·4		3·93	571
							23·7		3·97	543
									Mean	560

*Approximate value

TABLE V

OXYGEN CONSUMPTION IN RELATION TO SALINITY

Salinity : 30°/oo *Time : after 72 hrs.* *Rate of flow : 52 ml./min.*

Exp. No.	No. of prawns used	Total weight (dry) of prawns in gm.	Period of exp.	Inflow			Outflow			Rate of oxygen consumption in c. mm./gm./hr.
				Mean temp °C	pH*	Mean oxygen eontent in ml./L. at N.T.P.	Mean temp °C (for each hr.)	pH*	Mean oxygen content in ml./L. at N.T.P. (for each hr.)	
1	3	2·641	4 hrs.	28·1	8·3	4·76	26·3	8·3	4·28	567
							27·1		4·25	603
							25·6		4·32	520
							26·7		4·37	461
2	3	2·829	4 hrs.	27·4	8·1	4·62	25·6	8·1	4·04	640
							26·3		4·15	518
							25·9		7·17	496
							26·3		4·11	563
3	3	4·656	4 hrs.	26·9	8·5	4·71	26·0	8·5	4·02	462
							25·6		3·87	563
							25·8		3·84	583
							25·5		3·98	489
4	3	4·809	4 hrs.	28·3	8·7	4·80	25·6	8·7	4·01	513
							25·7		4·10	454
							26·0		3·98	532
							25·8		3·94	558

*Approximate value Mean 533

state is reached (Panikkar, 1941). It was thought desirable to find out how the rate of oxygen consumption is affected by this slow process of acclimatization and hence experiments similar to those described above were conducted using prawns which had been kept in sea water diluted to salinity 30 °/₀₀ for different periods, viz., sixteen, twentyfour, fortyeight and seventy-two hours. The results obtained in these experiments are given in Tables II, III, IV and V. It will be noticed that the average rate of oxygen consumption after sixteen hours' keeping in the medium is 563 c.mm. per gm. per hour (Table II), and that this rate is approximately 20% higher than that obtained for prawns kept in an identical medium for four hours. When the period is increased to twentyfour hours (See Table III) the average rate is found to be 599 c.mm. per gm. per hour. This rate is slightly higher than after sixteen hours, but the difference is not significant. It will be seen from Table IV that the average rate of oxygen consumption after acclimatizing the prawns for a period of fortyeight hours (560 c.mm. per gm. per hour) is also nearly the same as after sixteen and twentyfour hours. The rate of oxygen consumption is found to be apparently constant even after increasing the period to seventytwo hours, as will be seen from the experimental data presented in Table V, which show an average rate of 533 c.mm. per gm. per hour. These results show an apparent correlation between the rate of oxygen consumption and the process of osmoregulation in the prawn.

The above presented results indicate that the rate of oxygen consumption of the prawn in a medium of salinity 30 °/₀₀ (approximately cent percent sea water) becomes steady after sixteen hours of transfer from the environmental medium of salinity 20 °/₀₀ and that it continues to remain so even after prolonged stay for twentyfour, fortyeight and seventytwo hours. Previous work on the effect of salinity changes upon respiration rates for different intervals of time appears to be meagre. It has been observed that in the triclad worm *Gunda ulvae*, when the medium is changed to a hypertonic one, the rate of respiration increases rapidly and remains constant for an indefinite period (Beadle, 1931). In the amphipod *Gammarus chevreuxi*, the reduction in respiration rate observed, when the animals were transferred to hypertonic solutions remained constant even after twelve days (Lowenstein, 1935). On the other hand, in the polychaete worm *Nereis diversicolor* the rate of oxygen consumption rises comparatively quicker to the maximum level

when transferred to hypotonic medium, but falls again after reaching the maximum, (Beadle 1931).

Rate of oxygen consumption in sea water of salinity 20 °/₀₀

Dilute sea water of salinity 20 $^\circ/_{00}$ was used for the present set of experiments and this being similar to the environmental medium, only one set of observations was thought necessary. Table VI gives the experimental data obtained in these experiments. Care was taken to keep all the experimental conditions (except salinity) constant and identical with those of earlier experiments. The results obtained show that the rate of oxygen consumption in this medium ranges between 635 and 857 c.mm. per gm. per hour. The average rate of 757 c.mm. per gm. per hour is about 60% greater than in a medium of salinity 30 $^\circ/_{00}$. It may appear then that when these animals are transferred from the normal medium to one nearly cent per cent sea water a marked decrease in rate of oxygen consumption takes place, which remains more or less constant after about sixteen hours.

Rate of oxygen consumption in media of salinities 15 $^\circ/_{00}$ and 10 $^\circ/_{00}$

The results obtained in four sets of observations (See Table VII) show that the rate of oxygen consumption in a medium of salinity 30 $^\circ/_{00}$ is between 772 and 959 c.mm. per gm. per hour, with an average of 878. It will be noted that this rate is about 16% higher than that in a medium of salinity 20 $^\circ/_{00}$, and that these observations suggest a relationship between the osmoregulatory activity taking place in hypotonic medium and the oxygen consumption. Further experiments were conducted with sea water diluted to salinity 10 $^\circ/_{00}$. The effect of time on the rate of oxygen consumption of the prawn in this medium has also been studied.

Experiments conducted with prawns that had been kept in the medium of salinity 10 $^\circ/_{00}$ for four hours indicated that the average rate of oxygen consumption by the animal increases to about 1059 c.mm. per gm. per hour, the separate values ranging from 889 to 1287 (Table VIII). The average rate is about 20% higher than in the medium of salinity 15 $^\circ/_{00}$ (vide supra). The results presented in Table IX were obtained when prawns kept in an identical medium for sixteen hours were used for study. The average rate of about 1212 c.mm. per gm. per hour

TABLE VI

OXYGEN CONSUMPTION IN RELATION TO SALINITY

Salinity: 20°/oo *Time: after 4 hrs.* *Rate of flow: 52 ml/min.*

Exp. No.	No. of prawns used	Total weight (dry) of prawns in gm.	Period of exp.	Inflow: Mean temp °C	Inflow: pH*	Inflow: Mean oxygen content in ml./L. at N.T.P.	Outflow: Mean temp °C (for each hr.)	Outflow: pH*	Outflow: Mean oxygen content in ml./L. at N.T.P. (for each hr.)	Rate of oxygen consumption in c. mm./gm/hr.
1	3	2·622	4 hrs.	27·0	8·4	4·44	26·0	8·4	3·79	774
							26·1		3·75	821
							26·1		3·72	857
							26·3		3·87	678
2	3	3·342	4 hrs.	27·3	8·2	4·33	26·4	8·2	3·65	635
							26·5		3·48	794
							26·3		3·55	728
							26·0		3·57	710
3	3	3·759	4 hrs.	28·5	8·0	4·10	25·8	8·0	3·12	814
							23·7		3·27	689
							25·1		3·21	739
							25·2		3·08	847
4	3	3·930	4 hrs.	26·1	8·1	4·21	25·8	8·1	3·27	746
							25·3		3·20	802
							24·8		3·31	715
							24·1		3·24	770
									Mean	757

*Approximate value

TABLE VII

OXYGEN CONSUMPTION IN RELATION TO SALINITY

Salinity : 15°/oo *Time : after 4 hrs.* *Rate of flow: 52 ml./min.*

Exp. No.	No. of prawns used	Total weight (dry) of prawns in gm.	Period of exp.	Inflow			Outflow			Rate of oxygen consumption in c. mm./gm./hr.
				Mean temp °C	p*	Mean oxygen content in ml./L. at N.T.P	Mean temp °C (for each hr.)	pH*	Mean oxygen content in ml./L. at N.T.P. (for each hr.)	
1	3	3·264	4 hrs.	26·5	8·2	4·62	26·0	8·2	3·66	918
							24·9		3·74	841
							25·6		3·70	880
							25·3		3·62	956
2	3	3·483	4 hrs.	27·7	8·2	4·50	26·5	8·2	3·47	923
							25·8		3·51	887
							26·2		3·48	914
							25·7		3·55	851
3	3	3·639	4 hrs.	27·4	8·0	4·30	26·3	8·0	3·30	857
							25·8		3·36	806
							25·4		3·26	892
							25·9		3·40	772
4	3	3·840	4 hrs.	26·9	8·4	4·54	25·7	8·4	3·41	918
							24·9		3·36	959
							23·8		3·47	870
							25·5		3·55	804

* Approximate value Mean 878

TABLE VIII

OXYGEN CONSUMPTION IN RELATION TO SALINITY

Salinity : 10°/oo *Time : after 4 hrs.* *Rate of flow : 52 ml./min.*

Exp. No.	No. of prawns used	Total weight (dry) of prawns in gm.	Period of exp.	Inflow: Mean temp °C	Inflow: p^{H*}	Inflow: Mean oxygen content in ml./L. at N.T.P.	Outflow: Mean temp °C (for each hr.)	Outflow: p^{H*}	Outflow: Mean oxygen content in ml./L. at N.T.P. (for each hr.)	Rate of oxygen consumption in c.mm./gm./hr.
1	3	3·075	4 hrs.	27·1	8·2	4·51	26·4	8·2	3·51	1015
							25·5		3·59	934
							26·2		3·43	1096
							24·8		3·39	1137
2	3	3·723	4 hrs.	26·3	8·6	4·38	25·1	8·6	3·32	889
							25·6		3·18	1006
							26·0		3·29	914
							25·7		3·15	1031
3	3	3·774	4 hrs.	26·8	8·3	4·17	26·4	8·3	2·78	1149
							25·7		2·66	1248
							24·3		3·05	926
							24·8		2·90	1050
4	3	3·951	4 hrs.	28·5	8·1	4·25	27·2	8·1	2·82	1129
							26·4		2·79	1153
							25·0		2·62	1287
							26·1		3·01	979
										Mean 1059

*Approximate value

TABLE IX

OXYGEN CONSUMPTION IN RELATION TO SALINITY

Salinity: 10°/oo *Time: after 16 hrs.* *Rate of flow: 52 ml/min.*

Exp. No.	No. of prawns used	Total weight (dry) of prawns in gm.	Period of exp.	Inflow			Outflow			Rate of oxygen consumption in c. mm/gm./hr.
				Mean temp °C	pH.*	Mean oxygen content in ml/L. at N.T.P.	Mean temp °C (for each hr.)	pH.*	Mean oxygen content in ml./L. at N.T.P. (for each hr.)	
1	3	2·565	4 hrs.	28·3	8·5	4·36	26·0	8·5	3·42	1144
							26·2		3·48	1070
							26·1		3·34	1241
							25·7		3·23	1375
2	3	3·369	4 hrs.	26·6	8·0	4·15	25·4	8·0	2·79	1260
							25·7		2·92	1140
							25·7		2·83	1223
							25·6		2·98	1083
3	3	4·074	4 hrs.	27·5	8·2	4·42	26·4	8·2	2·96	1119
							26·2		2·88	1180
							25·9		2·79	1248
							25·8		2·70	1317
4	3	4·425	4 hrs.	28·5	8·3	4·32	27·0	8·3	2·58	1227
							26·5		2·43	1332
							26·6		2·54	1255
							26·8		2·64	1185

*Approximate value — Mean 1212

is definitely greater than that after four hours (Table VIII). Similar determinations made after increasing the period to twentyfour and fortyeight hours showed that the rates remain more or less stationary, at averages of 1254 and 1225 c.mm. per gm. per hour (See Tables X and XI). Hence it is evident that in this hypotonic medium the rate of oxygen consumption becomes steady after about sixteen hours.

From the experiments described above, it appears that the rate of oxygen consumption is influenced by changes in salinity of the medium, the rate being highest in sea water of salinity $10\ ^{\circ}/_{00}$ and lowest in that of $30\ ^{\circ}/_{00}$. That this variation in oxygen consumption may be due to changes in salinity only is suggested by the fact that all other conditions were kept similar in the course of observations and that the movements of the prawn were reduced to the barest minimum in the respiration chamber. Prawns of medium size only were chosen for the experiments, and slight differences in the size do not seem to have affected the rssults appreciably. The differences in hydrogen-ion concentration noted also do not affect the results (Well and Pantin, 1931; Lowenstein, 1935). Hence it is reasonable to infer that changes in the respiratory rate may be due to changes in salinity of the medium only and the consequent osmoregulatory activity.

It is known that when animals with osmoregulatory powers adjust themselves to varying salinity conditions of the external media, energy is expended and it is natural to expect corresponding variations in oxygen consumption (Beadle, 1931; Lowenstein, 1935; Krogh, 1939; Hopkins, 1949). *Gammarus chevreuxi* taken from a medium of salinity equivalent to 25% sea water was used by Lowenstein (1935) for study and it was shown that the rate of oxygen consumption increases when the salinity is lowered. In the copepod *Calanus finmarchicus* also such a condition has been observed, (Marshall, Nicholls and Orr, 1935). Supporting evidences are found in studies on polychaete worms. Tarussov (1927) was the first to state that the respiratory rate of *Nereis diversicolor* is increased in hypotonic and decreased in hypertonic sea water. *Carcinus maenas* shows increased respiratory rate in a diluted medium (Schwabe, 1933; Krogh, 1939). Beadle (1931) also confirmed this to a certain extent and observed that the differences in the amount of increase in respiratory rate are more marked in water containing only 16.6% sea water than in water containing 25% sea water. In the present observations on *Penaeus*

TABLE X

OXYGEN CONSUMPTION IN RELATION TO SALINITY

Salinity: 10°/oo *Time: after 24 hrs.* *Rate of flow: 52 ml./min.*

				Inflow			Outflow			
Exp. No.	No. of prawns used	Total weight (dry) of prawns in gm	Period of exp.	Mean temp °C	pH*	Mean oxygen eontent in ml /L. at N.T.P.	Mean temp °C (for each hr.)	pH*	Mean oxygen content in ml./L. at N.T.P. (far each hr.)	Rate of oxygen consumption in c. mm./gm./hr.
1	3	2·982	4 hrs.	28·3	8·1	4·52	27·2	8·1	3·54	1025
							27·3		3·41	1161
							27·0		3·32	1256
							26·8		3·19	1392
2	3	3·195	4 hrs.	28·2	7·8	4·67	27·7	7·8	3·36	1279
							27·5		3·15	1485
							27·7		3·54	1104
							27·2		3·29	1348
3	3	3·510	4 hrs.	27·5	8·3	4·60	26·5	8·3	3·05	1378
							26·2		3·23	1218
							26·3		3·14	1298
							27·0		3·45	1022
4	3	4·017	4 hrs.	27·8	8·2	4·47	26·8	8·2	2·82	1281
							26·0		2·64	1422
							26·4		2·97	1165
							27·1		2·88	1235

* Approximate value

Mean 1254

TABLE XI

OXYGEN CONSUMPTION IN RELATION TO SALINITY

Salinity : 10°/oo *Time : after 48 hrs.* *Rate of flow : 52 ml /min.*

				Inflow			Outflow			
Exp. No.	No. of prawns used	Total weight (dry) of prawns in gm.	Period of exp.	Mean temp °C	pH*	Mean oxygen content in ml./L. at N.T.P.	Mean temp °C (for each hr.)	pH*	Mean oxygen content in ml./L. at N.T.P. (for each hr.)	Rate of oxygen consumption in c. mm./gm./hr.
1	3	3·402	4 hrs.	26·9	8·3	4·28	26·0	8·3	3·17	10.8
							26·3		2·96	1211
							26·4		3·11	1073
							26·1		2·84	1320
2	3	3·546	4 hrs.	27·8	8·6	4·20	26·8	8·6	2·63	1381
							26·6		2·95	1100
							25·8		2·85	1188
							26·2		2·72	1302
3	3	4·152	4 hrs.	28·1	8·0	4·44	27·4	8·0	2·78	1247
							27·0		2·64	1353
							26·8		2·95	1120
							26·5		3·02	1068
4	3	4·650	4 hrs.	28·3	8·2	4·45	27·6	8·2	2·64	1214
							27·1		2·46	1336
							26·6		2·25	1476
							26·7		2·68	1188
									Mean	1225

* Approximate value

indicus, it is evident that the increase in rate of oxygen consumption is more marked in water of salinity 10 °/₀₀ than in that of salinity 15 °/₀₀. However, Schwabe (1933) was unable to record any difference in the respiratory metabolism of *Eriocheir* in sea water of salinities 15 °/₀₀ and 32 °/₀₀ and also fresh water. Likewise, Schlieper (1931) has found decrease in respiration rate of *Gammarus locusta* when the salinity of the medium is increased to 32 °/₀₀ from 16 °/₀₀. But the rate has been shown to be almost the same in different concentrations of brackish water, increasing only in very dilute solutions. This led the author to believe that osmotic regulation occurs in the animal only in very dilute solutions. Bateman (1933) suggested that the latter observation might not be interpreted to mean that osmoregulatory activity takes place only at great dilution. Kreps (1929) found that in *Balanus* the respiration is independent of salinities from 12 °/₀₀ to 35 °/₀₀, but below 12 °/₀₀ the rate decreases. The absence of changes in the rate of oxygen consumption in some instances may be due to other related physiological processes like ionic regulation. It has also been suggested that the amount of energy spent during osmoregulatory activity might not be sufficiently large to show marked changes in respiratory rates (Krogh, 1939). The experiments of Schwabe (1933) on *Eriocheir* and *Potamobius* suggest that the increase in metabolic rate may not be exclusively due to the salt absorbing mechanism. It is known that the gill tissues are very active during osmoregulatory activity (Krogh, loc. cit). Pieh (1936) showed the relationship between the degree of swelling of the gills in *Carcinus*, *Mytilus* and *Eriocheir* and the respiration of the tissue. The gills of *Venus mercenaria* consume one third more of oxygen in sea water of density 1.015, than in natural sea water of density 1.025 (Hopkins, 1946).

As stated earlier, it is clear from the results obtained in the present study that a constant rate of oxygen consumption in the hypertonic medium of salinity 30 °/₀₀ is attained after a period of about sixteen hours. Constant rate in hypotonic medium also is attained after about sixteen hours. It may be supposed that the time thus taken to reach the constant rate is more or less related to the attainment of "steady state" of osmoregulatory activity. It has been shown by Panikkar (1941) that the time taken to reach the limits of osmotic changes are variable according the strength of the experimental medium and also the size of the individuals. He demonstrated that when *Leander* and *Palaemonetes* were transferred to hypotonic media, the osmotic pressure fell gradually for about fourteen to

twentyfour hours, varying according to the size of the individual, before "steady state" is attained. The experiments of Lowenstein (1935) on *Gammarus* showed that changes in oxygen consumption were constant throughout after the transfer, thereby showing that the constant rate is attained immediately.

The relation between the rate of oxygen consumption and the salinity of the medium is represented in Fig. 4. The graph shows the inversely proportional relationship between the two factors. In Fig. 5, the rate of oxygen consumption in media of salinities 10 °/oo and 30 °/oo is plotted against the time in the medium after transfer from the environmental medium. The graph shows the gradual attainment of constant respiratory rates in hypertonic and hypotonic media.

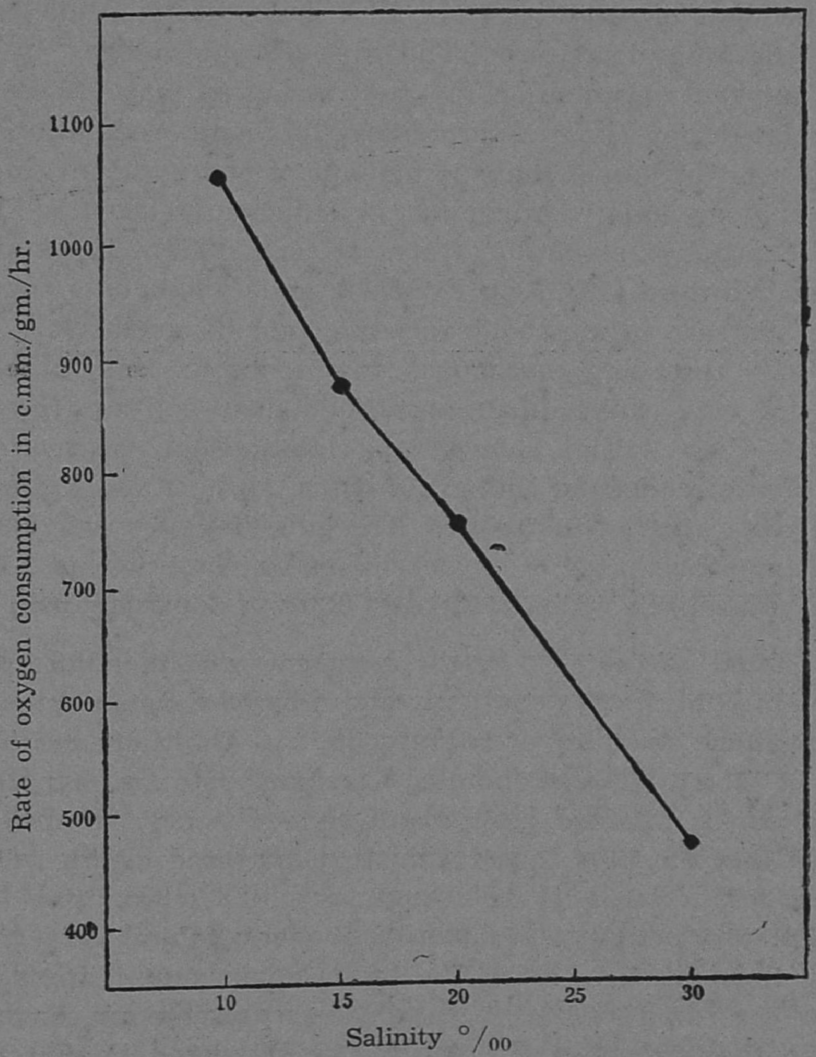

Fig. 4.

Curve illustrating the influence of salinity on the rate of oxygen consumption. (Time, after 4 hours).

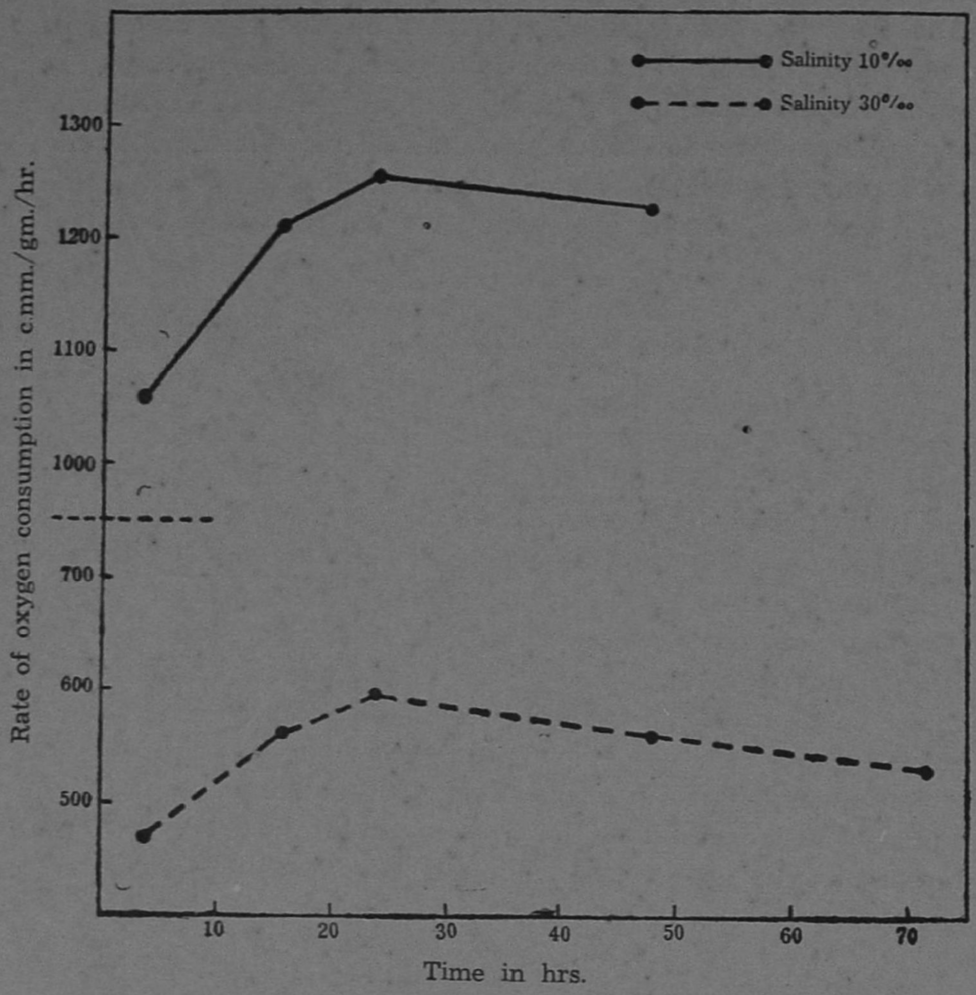

Fig. 5.
Curves illustrating the influence of time on the rate of oxygen consumption in hypotonic and hypertonic media.

Influence of Temperature on Oxygen Consumption

The lower and higher limits of temperature that the prawn could withstand were first determined before studying the influence of temperature between these limits. The mean from ten observations for each limit helped to fix the lower and upper limits at 14.8° and 43.2°C., respectively. The individual differences among the prawns experimented appeared slight. These limits, it must be admitted, have no absolute value since acclimatization may lead to temperature adaptation as shown by Hathaway (1927), Wells (1935) and Sumner and Doudoroff (1938) for other animals. The factors responsible for death by heat at the upper limit of temperature discussed by Fraenkel and Hopf (1940) also may be altered by adaptation to different habitats.

The influence of temperature on the prawn, as in any animal, can be best studied in a measurable way only with reference to the effect of temperature of the medium on the rate of oxygen consumption of the animal. Hence experiments were conducted to determine the rates of oxygen consumption in different temperature ranges above and below that of the normal habitat, keeping the salinity of the sea water constant at 20 °/oo.

Rate of oxygen consumption at 25·4°—27·8°C

The above temperature range is similar to that of the waters of the Cooum river, which is the natural habitat of the prawns used, at the time of study. As in the observations already described, three prawns were used for each experiment and the experimental proceedure also was indentical. But as the main factor in these experiments was temperature, water in the bath was constantly watched and its temperature adjusted within a narrow range. The p^H of the inflow and outflow water did not vary much, the range being 8.4 to 8.8. Determinations of oxygen content of water samples were made in duplicate and the rate of oxygen consumption expressed as c.mm. per gm. per hour are given in Table XII. The average rate of oxygen consumption of the prawn under the present conditions was found to be 737 c.mm. per gm. per hour.

Rate of oxygen consumption at 33·1—36·2°C

In order to see if increase of temperature of the medium would have any affect on the rate of oxygen consumption, the temperature of the bath was maintained at 41.8 to 44.2°C., so that the outflow water indicated temperatures between 33.1 and 36.2°C. The results obtained (Table XIII) show that the rate of oxygen consumption ranges from 867 to 1114 c.mm. per gm. per hour. It may be inferred from these results that the rise in temperature of the water in the respiration chamber increases the rate of oxygen consumption by the animals.

Rate of oxygen consumption at 19·5° — 22·5°C

With a view to see if the rate of oxygen intake differs at a temperature lower than that of the natural environment, the following experiments were performed by adding ice-cooled water to the bath, so that its temperature varied between 13.25° and 17.4 °C. In this way the temperature of the outflow water was reduced to 19.5 — 22.5 °C. The experimental data under

TABLE XII

OXYGEN CONSUMPTION IN RELATION TO TEMPERATURE

Temperature : 25·4° — 27·8°C Time : after 4 hrs.
Salinity : 20°/oo Rate of flow : 52 ml./min.

				Inflow			Outflow			
Exp. No.	No of prawns used	Total weight (dry) of prawns in gm.	Period of exp.	Mean temp °C	p^H.	Mean oxygen content in ml./L. at N.T.P.	Mean temp °C (for each hr.)	p^H.*	Mean oxygen content in ml./L. at N.T.P. (for each hr.)	Rate of oxygen consumption in c. mm./gm./hr.
1	3	2·454	4 hrs.	28·9	8·4	3·97	27·4	8·4	3·41	712
							27·8		3·44	674
							26·5		3·35	788
							26·2		3·33	814
2	3	3·825	4 hrs.	28·3	8·6	3·89	25·5	8·6	3·05	685
							26·2		3·12	628
							27·0		3·01	718
							26·5		3·06	677
3	3	3·945	4 hrs.	28·1	8·8	4·16	27·2	8·8	3·30	680
							27·6		3·24	728
							26·1		3·22	744
							25·4		3·15	799
4	3	5·010	4 hrs.	29·0	8·5	4·05	26·3	8·5	2·90	716
							27·5		2·81	772
							27·2		2·73	822
							27·5		2·70	841

*Approximate value Mean 737

TABLE XIII

OXYGEN CONSUMPTION IN RELATION TO TEMPERATURE

Temperature: *33·1°–36·2°C* *Time*: *after 4 hrs.*
Salinity: *20°/oo* *Rate of flow 52 ml./min.*

				Inflow			Outflow			
Exp. No.	No. of prawns used	Total weight (dry) of prawns in gm.	Period of exp.	Mean temp °C	pH*	Mean oxygen content in ml./L. at N.T.P.	Mean temp °C (for each hr.)	pH*	Mean oxygen content in ml./L. at N.T.P. (for each hr.)	Rate of oxygen consumption in c. mm./gm./hr.
1	3	1·569	4 hrs.	28·7	8·3	3·82	34·5	8·3	3·26	1114
							33·1		3·33	975
							35·1		3·30	1058
							34·8		3·34	955
2	3	3·240	4 hrs.	29·2	8·4	4·13	35·2	8·4	3·07	1021
							33·7		3·10	992
							34·8		3·02	1069
							36·0		3·12	973
3	3	3·804	4 hrs.	28·9	8·8	4·12	33·9	8·8	2·94	968
							34 8		2·82	1066
							35·9		3·02	902
							33·6		2·98	935
4	3	4·790	4 hrs.	28·5	8·7	4·28	35·3	8·7	2 89	906
							34·9		2·71	1023
							35·6		2·95	867
							36·2		2·65	1062
										Mean 993

*Approximate value

TABLE XIV

OXYGEN CONSUMPTION IN RELATION TO TEMPERATURE

Temperature: 19·5—22·5°C *Time: after 4 hrs.*
Salinity: 20°/oo *Rate of flow: 52 ml./min.*

Exp. No.	No. of prawns used	Total weight (dry) of prawns in gm.	Period of exp.	Inflow: Mean temp °C	Inflow: pH.*	Inflow: Mean oxygen content in ml/L. at N.T.P.	Outflow: Mean temp °C (for each hr.)	Outflow: pH.*	Outflow: Mean oxygen content in ml./L. at N.T.P. (for each hr.)	Rate of oxygen consumption in c. mm./gm./hr.
1	3	1·305	4 hrs.	28·6	8·5	3·67	21·6	8·5	3·45	526
							20·2		3·50	407
							20·8		3·48	454
							22·5		3·42	598
2	3	2·670	4 hrs.	28·0	8·4	3·52	19·8	8·4	3·05	549
							22·2		3·16	421
							21·1		3·01	596
							20·6		3·07	526
3	3	3·420	4 hrs.	29·0	8·2	3·60	21·8	8·2	3·12	438
							19·5		3·17	392
							20·4		3·20	365
							21·5		3·18	383
4	3	3·468	4 hrs.	29·4	8·6	3·75	22·7	8·6	3·29	414
							21·5		3·21	486
							21·2		3·38	333
							20·0		3·33	378
										Mean 454

*Approximate value

this lower temperature condition, presented in Table XIV, show that the average rate of oxygen consumption is only 454 c.mm. per gm. per hour.

The movements made by the animals inside the respiration chamber were slight and since other factors like salinity, oxygen content etc., were all identical, it is believed that the higher rate at increased temperature and lower rate at reduced temperatures must be due to the effect of temperature changes only.

The influence of temperature on the rate of oxygen consumption is represented graphically in Fig. 6. From the tables as well as the graph it will be seen that the rate of oxygen consumption varies directly with the temperature of the medium. The rate of oxygen consumption of the animals, used to a range of 27—29 °C., when subjected to ranges round 21 °C. is lower and round 35 °C. is higher proportionate to the magnitude of the temperature difference. At about 21 °C., which is below the normal, the rate of oxygen consumption falls far below and there is a slackening of the metabolic rate due to fall in temperature of the external medium. However, when the temperature is increased to 35 °C. the prawn consumes more of oxygen indicating an increase in the metabolic rate. In both cases it will be seen that the decrease or increase of oxygen consumption is directly proportional to the temperature decrease or increase. It will be seen from the graph that continued lowering of temperature will reduce the oxygen consumption below the minimum required for life. Increase of temperature will not be possible beyond the lethal limit, when the rate of metabolism will decrease due to death of the tissues.

Earlier studies on temperate and arctic marine animals showed that in many forms increase or decrease in temperature of the external medium, above and below the normal, is accompanied by increase or decrease in rate of oxygen consumption. It has been demonstrated that in the copepod *Calanus finmarchicus* the rate of oxygen consumption increases directly proportional to the rise in temperature (Marshall *et al*, 1935). Identical results have been obtained for *Carcinus maenas* also (Capraro, 1939). The studies of Fox (1936) and Fox and Wingfield (1937) indicate that the crustaceans collected from Plymouth show a higher rate of oxygen consumption than related species from the colder zone of Sweden. Among the arthropods surveyed by Edwards (1946), it was found that all except the sand crab *Emerita talpoida* became less active in lower tem-

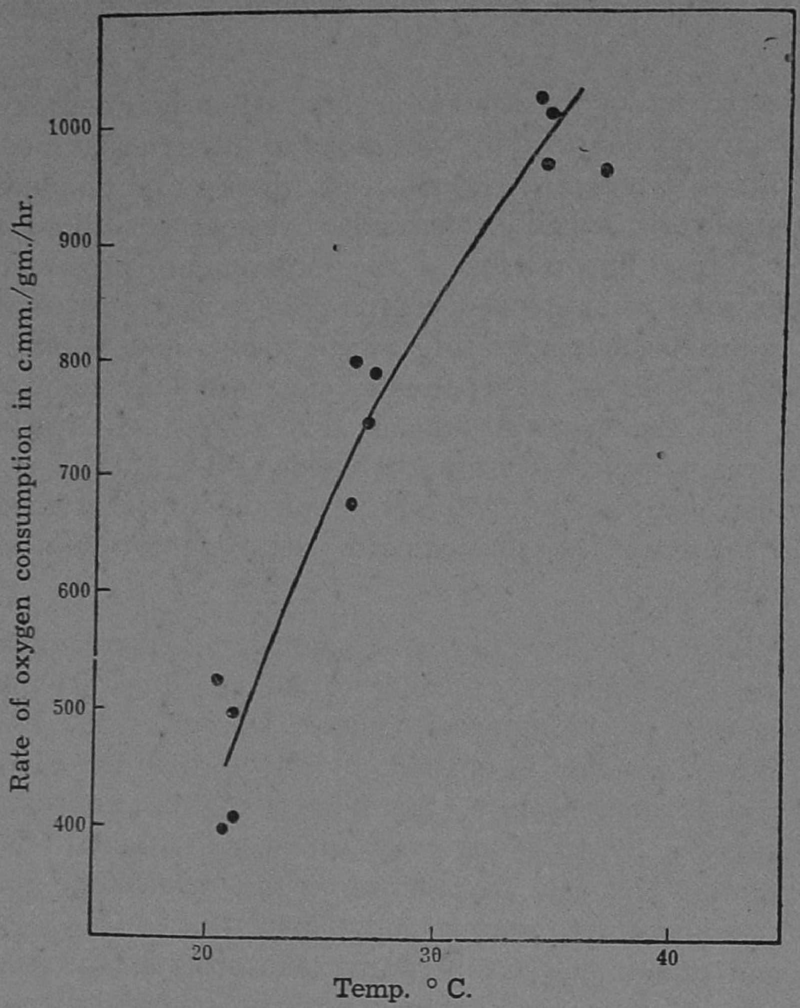

Fig. 6.

Curve illustrating the influence of temperature on the rate of oxygen consumption.

peratures and that an internal adjustment in metabolic rate to offset the effects of cold was noticed in *Emerita*. However, the rate of oxygen consumption of this animal also increases with rise in temperature of the external medium (Edwards and Irving, 1943). On the other hand, instances have been recorded where increased temperature of the medium affects the respiration in a very different manner. Sparch (1936) found that the rate of oxygen consumption in lamellibranchs collected from Denmark and Greenland was higher than in related forms collected from the warmer Mediterranean region. The rate of oxygen consumption of *Astacus fluviatilis* has recently been shown to be constant for the range 5-15 °C. (Schlieper, 1950), although earlier studies by Brunow (1911) indicated that the rate is about four times higher at 15 °C. than at 5 °C. during a period of six to ten hours. It may also be mentioned that the

effects of temperature vary with different animals (Krogh, 1941).

A comparison of the studies on the relation between oxygen consumption and temperature variations of the external medium carried out in temperate regions, with the results obtained in the present work, suggest that under warmer conditions it is not very close. The results of the experiments presented in this paper show that oxygen consumption may decrease more rapidly in the lower temperature ranges than would be expected in temperate animals. This greater decrease may be due to the fact that the tropical animals live near their maximum limits of temperature toleration (Mayer, 1914, 1918), so that any further decrease in temperature of the external medium than that the animal is accustomed to, reduces the rate of metabolism considerably.

The Van't Hoff's law has been applied in several instances to changes in respiratory exchange of animals due to temperature differences of the external medium (Krogh, 1916; Rogers, 1938). When this law is applied, it follows that for each ten degree rise in temperature, the rate of oxygen consumption should increase by twice or even thrice the original. Krogh (1916) studied this law in relation to the respiratory rate in different temperature ranges and noted that "Q_{10}" varied greatly and that at low temperatures the observed increase in metabolism due to increase of temperature is larger than what the theory would demand, while at higher temperatures it is less so. On this basis he supported the view that the rule of Van't Hoff cannot be applied indiscriminately. The studies of Belehradek (1930) and Marshall *et al* (1935) also have confirmed this view. The results of the experiments described in this paper for a high temperature range of about 21 — 35 °C. show that "Q_{10}" decreases with increasing temperature. As suggested by Krogh (1916) this may be due to the fact that the oxygen pressure in the body tissues is a limiting factor in the reactions.

Influence of Hydrogen-ion Concentration on Oxygen Consumption

In order to study the influence of hydrogen-ion concentration on the rate of oxygen consumption, three different sets of experiments were conducted. The medium used was sea water diluted to salinity 20 ‰ and the p^H was reduced or increased as described (vide material and methods). The p^H of the

water in the three sets of experiments were in the range 7.5 to 8.0, 6.6 to 7.2 and 8.8 to 9.4 respectively. During the course of the experiments the p^H of the outflow showed changes and exact readings could not be taken due to lack of glass electrodes. However, these changes do not affect the rate of oxygen consumption as may be seen from the results obtained.

Rate of oxygen consumption at p^H 7·5 — 8·0

As in the previous experiments, three prawns were introduced into the respiration chamber and the outflow water samples were analysed at the end of each hour. The experimental data are presented in Table XV. The p^H values were 7.5, 8.0, 7.6 and 7.8 respectively for the four experiments. The results obtained show that the rate of oxygen consumption ranges between 642 and 820 c.mm. per gm. per hour, with the average at 745. The other factors like temperature, oxygen content and salinity were kept identical for all the experiments.

Rate of oxygen consumption at p^H 6·6 — 7·2

Identical experiments were conducted after reducing the p^H of the water to 6.6, 7.2, 6.8 respectively. The results obtained in the four experiments (See Table XVI) show that the average rate of oxygen consumption is 785 c.mm. per gm. per hour. The separate values range from 677 to 919 c.mm. per gm. per hour. It is seen that the average rate is almost the same as in the medium having hydrogen-ion concentration of 7.5 — 8.0 in the p^H scale.

Rate of oxygen consumption at p^H 8·8 — 9·4

In this set of experiments the p^H of the inflow water was increased to 9.1, 8.8, 9.2 and 9.4. As usual three prawns were used for each four hour period and the analyses of the inflow and outflow water (Table XVII) show that the average rate of oxygen consumption is 731 c.mm. per gm. per hour, which rate approximates the rates in media having lower p^H values (See Table XV and XVI).

It may be suggested from these observations that the changes in hydrogen-ion concentration from p^H 6.6 to 9.4 have no detectable effect on the rate of oxygen consumption of the prawn, (Fig. 7). Whatever differences that are noticed in the results are all within the limits of experimental variation only. A similar observation on the respiration of the copepod *Calanus*

TABLE XV

OXYGEN CONSUMPTION IN RELATION TO p^H

p^H : 7·5—8 0 *Time: after 4 hrs.*
Salinity 20°/oo *Rate of flow: 52 ml./min.*

Exp. No.	No. of prawns used	Total weight (dry) of prawns in gm.	Period of exp.	Inflow			Outflow			Rate of oxygen consumption in c mm./gm./hr.
				Mean temp °C	p^H*	Mean oxygen content in ml./L. at N.T.P.	Mean temp °C (for each hr.)	p^H*	Mean oxygen content in ml./L. at N.T.P. (for each hr.)	
1	3	3·363	4 hrs.	27·8	7·5	4·22	26·4	7·7	3·35	807
							26·5		3·40	761
							25·7		3·47	696
							25·3		3·38	779
2	3	3·501	4 hrs.	26·7	8·0	4·44	26·1	8·1	3·61	740
							25·8		3·72	642
							24·9		3·55	793
							26·3		3·68	677
3	3	3·690	4 hrs.	28·1	7·6	4·39	27·1	7·6	3·58	685
							25·7		3·42	820
							26·4		3·48	770
							26·1		3·52	736
4	3	5·043	4 hrs.	28·5	7·8	4·27	27·8	8·1	3·16	687
							27·2		3·02	773
							26·5		2·98	798
							27·6		3·05	755
										Mean 745

*Approximate value

TABLE XVI

OXYGEN CONSUMPTION IN RELATION TO p^H

p^H: 6·6–7·2 *Time: after 4 hrs.*
Salinity: 20°/oo *Rate of flow: 52 ml./min.*

Exp. No.	No. of prawns used	Total weight (dry) of prawns in gm.	Period of exp.	Inflow: Mean temp °C	Inflow: p^H*	Inflow: Mean oxygen content in ml/L. at N.T.P.	Outflow: Mean temp °C (for each hr.)	Outflow: p^H*	Outflow: Mean oxygen content in ml./L. at N.T.P. (for each hr.)	Rate of oxygen consumption in c. mm./gm./hr.
1	3	2·784	4 hrs.	26·9	6·6	4·35	26·4	6·9	3·62	818
							26·3		3·57	874
							25·5		3·73	695
							24·7		3·53	919
2	3	3·159	4 hrs.	28·2	7·2	4·20	27·6	7·3	3·40	790
							27·0		3·53	677
							26·2		3·36	830
							26·5		3·35	840
3	3	3·492	4 hrs.	27·0	6·8	4·38	25·9	7·0	3·62	679
							26·7		3·48	804
							24·1		3·56	733
							25·8		3·45	831
4	3	3·849	4 hrs.	27·4	6·8	4·52	26·3	6·9	3·55	787
							23·2		3·63	722
							25·5		3·51	819
							26·5		3·60	746

*Approximate value

Mean 785

TABLE XVII

OXYGEN CONSUMPTION IN RELATION TO p^H

p^H : 8·8—9·4 *Time : after 4 hrs.*
Salinity : 20°/oo *Rate of flow : 52 ml./min.*

Exp. No.	No. of prawns used	Total weight (dry) of prawns in gm.	Period of exp.	Inflow: Mean temp °C	Inflow: p^H*	Inflow: Mean oxygen content in ml/L. at N.T.P.	Outflow: Mean temp °C (for each hr.)	Outflow: p^H*	Outflow: Mean oxygen content in ml./L. at N.T.P. (for each hr.)	Rate of oxygen consumption in c. mm./gm./hr.
1	3	2·775	4 hrs.	27·2	9·1	4·36	26·2	8·9	3·62	832
							25·0		3·58	877
							25·7		3·70	742
							26·5		3·72	720
2	3	2·982	4 hrs.	27·8	8·8	4·46	25·2	8·7	3·75	743
							24·9		3·69	806
							26·4		3·71	785
							26·8		3·76	732
3	3	3·030	4 hrs.	27·6	9·2	4·58	26·1	8·8	3·88	721
							25·8		3·98	618
							24·9		3·94	659
							25·3		3·81	793
4	3	3·465	4 hrs.	28·3	9·4	4·45	23·7	9·1	3·60	765
							25·0		3·72	657
							25·4		3·76	621
							25·1		3·75	630
									Mean	731

*Approximate value

finmarchicus has been made by Marshall *et al* (1935). Earlier, Helff (1928) showed that the rate of oxygen consumption in *Cambarus immunis* is not dependent on the changes in p^H of the external medium. On the other hand, many fishes have been found to be affected directly by increase or decrease in the p^H of the medium (Powers, 1922, 1930, 1932). This is supposed to be due to the influence of p^H of the external medium on the alkali reserve of the blood. Then it is likely that the prawn has the ability to vary the alkali reserve of its blood and hence the capacity to withstand variations in p^H of the water in which it lives.

Influence of Oxygen Tension on Oxygen Consumption

The effect of changes in oxygen content of the external medium on the oxygen consumption of *Metapenaeus monoceros* was studied by subjecting the animal to water of different oxygen concentrations. A slight change in the set up of the apparatus had to be effected for the following experiments. A thick layer of liquid paraffin was poured in the first aspirator (Fig. 1, 'A') also, to prevent air coming into contact with the water, the oxygen content of which was either increased or decreased (vide material and methods). Filtered sea water diluted to salinity 20 °/₀₀ was used and after making the necessary changes in oxygen content it was poured into the aspirator immediately. The oxygen estimations were made frequently during the course of the experiments.

Rate of oxygen consumption at oxygen concentrations above normal

The experimental data presented in Table XVIII show that the average rate of oxygen consumption in the medium having slightly higher oxygen tension than that of the environmental medium, is about 769 c.mm. per gm. per hour (Range 671—951 c.mm. per gm. per hour). As in the previous experiments, the separate values obtained are found to differ slightly from each other, but are all within the limits of experimental variations.

The data presented in table XIX show the rate of oxygen consumption of the prawn at a still higher oxygen concentration of the medium. The dissolved oxygen content of the medium used for the experiments varied from 6.72 to 7.74 ml./L. The results obtained show that in spite of this higher level of oxygen tension the respiratory rate is more or less constant, the average being 789 c.mm. per gm. per hour.

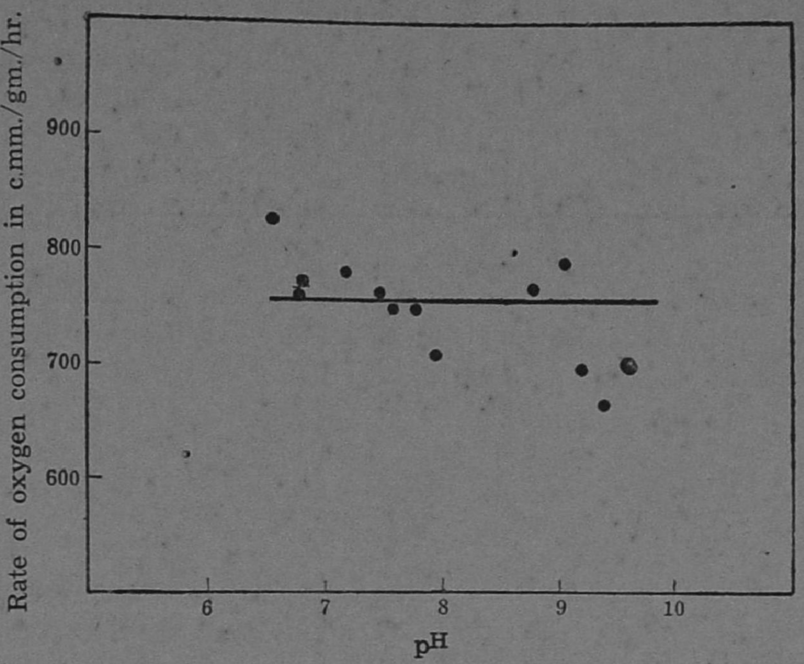

Fig. 7.
Curve illustrating the influence of p^H on the rate of oxygen consumption.

Rate of oxygen consumption at oxygen concentrations below normal

In the next set of experiments the medium was made to have lower than normal oxygen content. The results obtained are presented in Table XX. The separate values of rate of oxygen consumption range from 602 to 961 c.mm. gm. per hour, with an average of 795. It will be noted that in these media with oxygen content of 3.72—4.05 ml./L., there is no apparent increase or decrease in the rate of oxygen consumption, than in the environmental medium.

The rate of oxygen consumption of the prawn in a medium having very low oxygen tension was also studied and the experimental data are given in Table XXI. These observations revealed that when the dissolved oxygen concentration of the medium is reduced considerably, the rate of oxygen consumption of the animal decreases to a very low level. The oxygen content of the inflow water used in the experiments varied from 1.95 to 3.20 ml./L., and the results of the estimations show an average rate of 460 c.mm. per gm. per hour, the separate values being between 353 and 598 c.mm. per gm. per hour.

The effect of the changes in oxygen tension on the rate of oxygen consumption of the prawn is represented graphically in Fig. 8. The results of the experiments indicate that the rate

TABLE XVIII

OXYGEN CONSUMPTION IN RELATION TO OXYGEN TENSION

Oxygen content: 4·54—4·92 ml./L. Time: after 4 hrs.
Salinity: 20°/oo Rate of flow: 52 ml./min.

Exp. No.	No. of prawns used	Total weight (dry) of prawns in gm.	Period of exp.	Inflow: Mean temp °C	Inflow: pH*	Inflow: Mean oxygen content in ml./L, at N.T.P.	Outflow: Mean each °C (for each hr.)	Outflow: pH*	Outflow: Mean oxygen content in ml./L, at N.T.P. (for each hr.)	Rate of oxygen consumption in c. mm./gm./hr.
1	3	2·487	4 hrs.	27·5	8·5	4·92	27·1	8·5	4·36	703
						4·70	26·0		4·08	778
						4·72	26·5		4·14	728
						4·65	26·2		3·97	853
2	3	2·952	4 hrs.	25·8	8·2	4·82	24·5	8·2	4·18	677
						5·54	25·2		3·75	825
						4·62	25·0		3·72	951
						4·55	25·3		3·90	687
3	3	3·675	4 hrs.	26·5	8·0	4·85	25·6	8·0	4·06	671
						4·80	24·2		3·96	713
						4·81	23·6		4·00	688
						4·85	24·9		3·92	790
4	3	4·221	4 hrs.	27·1	8·4	4·72	26·7	8·4	3·54	872
						4·64	26·2		3·48	858
						4·62	26·4		3·51	821
						4·75	26·8		3·81	695

*Approximate value Mean 769

TABLE XIX

OXYGEN CONSUMPTION IN RELATION TO OXYGEN TENSION

Oxygen content : 6·72–7·74 ml./L. Time after 4 hrs.
Salinity : 20°/oo Rate of flow : 52 ml./min.

Exp. No.	No. of prawns used	Total weight (dry) of prawns in gm.	Period of exp.	Inflow			Outflow			Rate of oxygen consumption in c. mm /gm./hr.
				Mean temp °C	H*	Mean oxygen content in ml./L. at N.T.P.	Mean temp °C (for each hr.)	pH*	Meaa oxygen content in ml./L. at N.T.P. (for each hr.)	
1	3	2·653	4 hrs.	28·2	8·4	7·12	27·4	8·4	6·37	881
						6·84	25·8		6·16	798
						6·72	26·7		6·08	752
						7·05	26·5		6·46	693
2	3	3·528	4 hrs.	27·4	8·1	7·42	26·2	8·1	6·34	955
						7·31	24·6		6·45	761
						7·12	25·6		6·38	655
						7·06	25·8		6·23	734
3	3	4·713	4 hrs.	26·6	8·0	7·73	24·4	8·0	6·48	828
						7·74	25·0		6·44	861
						7·56	24·8		6·59	642
						7·43	24·1		6·20	814
4	3	4·842	4 hrs.	27·0	8·3	7·55	26·3	8·3	6·25	838
						7·32	26·5		6·16	748
						7·30	25·7		5·95	870
						7·25	25·2		6·03	786
									Mean	789

*Approximate value

TABLE XX

OXYGEN CONSUMPTION IN RELATION TO OXYGEN TENSION

Oxygen content: 3·72—4·05 ml./L. Time: after 4 hrs.
Salinity: 20°/oo Rate of flow: 52 ml./min.

Exp. No.	No. of prawns used	Total weight (dry) of prawns in gm.	Period of exp.	Inflow: Mean temp °C	Inflow: pH*	Inflow: Mean oxygen content in ml/L. at N.T.P.	Outflow: Mean temp °C (for each hr.)	Outflow: pH*	Outflow: Mean oxygen content in ml./L. at N.T.P. (for each hr.)	Rate of oxygen consumption in c. mm./gm./hr.
1	3	3·213	4 hrs.	27.3	8·0	3·82	26·7	8·0	2·83	961
						3·85	25·5		2·88	942
						3·87	26·2		3·04	806
						3·80	27·0		3·18	602
2	3	4·113	4 hrs.	26·4	8·4	3·94	25·5	8·4	2·87	812
						3·90	24·7		2·78	850
						3·95	24·2		2·98	736
						3·98	25·5		2·92	804
3	3	4·452	4 hrs.	27·0	8·3	3·72	26·3	8·3	2·50	855
						3·87	26·0		2·83	729
						3·95	25·4		2·75	841
						3·85	24·5		2·68	820
4	3	4·890	4 hrs.	25·8	8·1	4·01	25·1	8·1	3·02	632
						4·05	24·7		2·96	696
						3·98	25·2		2·72	802
						3·94	25·3		2·64	829
									Mean	795

*Approximate value

TABLE XXI

OXYGEN CONSUMPTION IN RELATION TO OXYGEN TENSION

Oxygen content : 1·95—3·20 ml./L. *Time after 4 hrs.*
Salinity : 20°/oo *Rate of flow : 52 ml./min.*

Exp. No.	No. of prawns used	Total weight (dry) of prawns in gm.	Period of exp.	Inflow			Outflow			Rate of oxygen consumption in c. mm./gm./hr.
				Mean temp °C	pH*	Meau oxygen content in ml./L. at N.T.P.	Mean temp °C (for each hr.)	pH*	Mean oxygen content in ml./L. at N.T.P. (for each hr.)	
1	3	2·298	4 hrs.	26·3	8·5	2·42	24·9	8·5	2·01	557
						2·73	23·7		2·47	353
						2·95	24·2		2·51	598
						2·47	24·5		2·09	516
2	3	3·282	4 hrs.	27·1	8·3	2·57	26·8	8·3	2·06	485
						3·04	26·1		2·58	437
						2·30	25·3		1·71	561
						2·15	25·8		1·73	399
3	3	3·324	4 hrs.	28·0	8·6	2·64	27·0	8·6	2·08	526
						2·90	24·7		2·46	413
						3·20	25·6		2·82	357
						2·52	24·0		2·04	451
4	3	4·320	4 hrs.	25·7	8·4	2·25	24·5	8·4	1·62	455
						2·58	23·1		2·00	419
						1·95	23·8		1·44	368
						2·60	24·3		1·95	470

*Approximate value

Mean 460

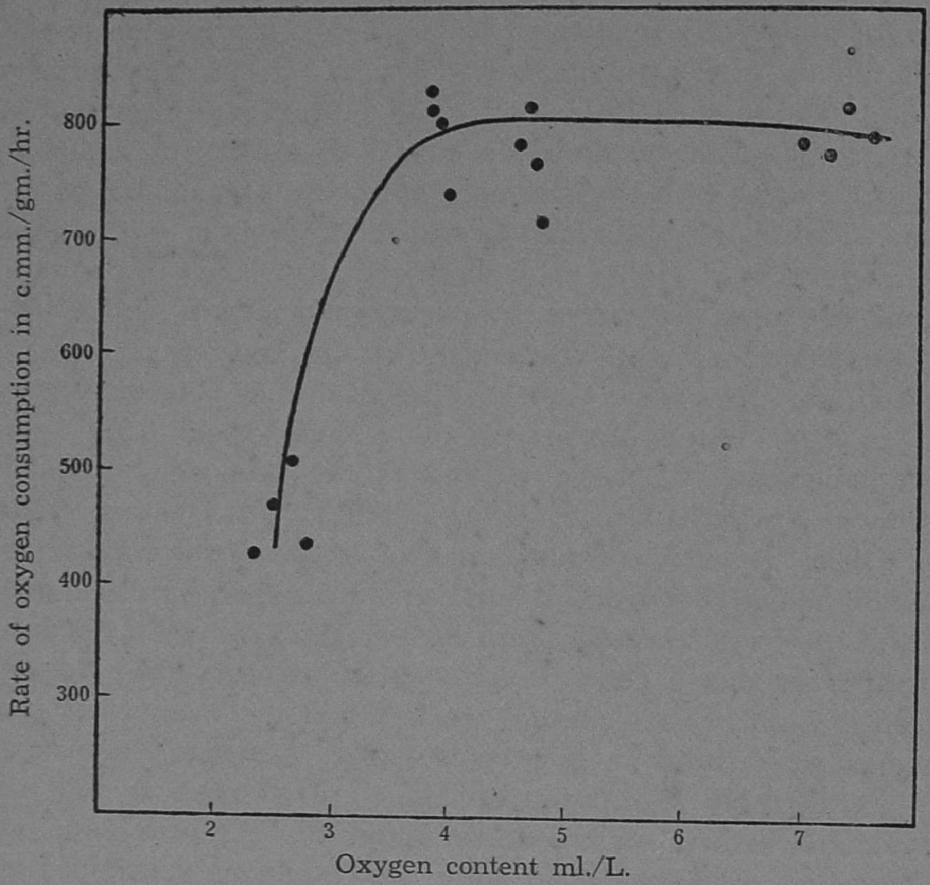

Fig. 8.

Curve illustrating the influence of oxygen tension on the rate of oxygen consumption.

of oxygen consumption of *Metapenaeus monoceros* is apparently unaffected by oxygen concentrations above about 3 ml./L. On the other hand, very low levels of oxygen tension, viz., below about 3 ml./L. reduce the rate of oxygen consumption considerably, which may be due to the fact that below the limit of respiratory regulation the animals are unable to tolerate the very low oxygen tension (Helff, 1928). Results identical with those described above have been obtained for other crustaceans also. It has been shown that in *Carcinus maenas* and *Scyllarus latus* the rate of oxygen consumption does not differ for wide ranges of oxygen tension of the medium (Henze, 1910). The same author concluded that in several cold-blooded animals the rate of metabolism is independent of the oxygen tension of the environment. The prawn *Palaemonetes* is able to tolerate changes in oxygen tension of the medium till the latter is lowered to about fifty per cent air saturation (Amberson *et al*, 1924). Similarly, the crayfish *Cambarus* is able to maintain constant rates till the saturation is reduced to about twentyfive per cent

(Helff, 1928; Hiestand, 1931). The copepod *Calanus finmarchicus* is apparently unaffected by higher oxygen tension of the water, but in concentrations below 3 ml./L., respiratory adjustment is not possible (Marshall *et al*, 1935). In *Pugettia*, at higher oxygen concentrations the rate of respiration is unaffected, but at lower levels it decreases markedly (Weymouth *et al*, 1944). The above observations are all in contrast with those of Amberson *et al* (1924) who showed that the oxygen cosumption of *Homarus americanus*, *Limulus polyphemus* and *Callinectis sapidus* is directly dependent on the oxygen tension of the medium. The same kind of relationship has been shown to exist in *Cancer* and *Eupagurus* also (Moore *et al*, 1912). A direct dependence of these two factors has been demonstrated in *Astacus fluviatilis* also by Brunow (1911), but Hee and Bonnetn (1925) showed constant rates of oxygen consumption in the same animal, between oxygen content of 5 and 8 ml./L., and also that the rate increased with further rise of oxygen tension. So it appears that a generalization on the relationship between oxygen tension of the external medium and the rate of oxygen consumption of the animals is not possible, as such relation may vary considerably in different animals. It is possible that in the prawn which is an active swimmer, the gill area exposed and the volume of respiratory water flowing being constant, the lower concentration of oxygen in the medium affect the rate of oxygen consumption, whereas, higher concentrations may not have any effect. It is also possible that further experiments in this direction may reveal that different animals have different basic needs of oxygen.

Summary

1. A study of the relation between the rate of oxygen consumption of *Metapenaeus monoceros* Fab., and the salinity, temperature, pH and oxygen content of the water in which it lives has been made.

2. It has been shown that the oxygen consumption of the prawn varies inversely proportional to changes in salinity of the medium. Constant rates of oxygen consumption are attained in hypotonic and hypertonic media after a period of about sixteen hours.

3. The rate of oxygen consumption changes directly proportional to the changes in temperature of the medium. The rate is determined at the temperature range of the natural

environment (25.4 — 27.8 °C.), as well as at a range lower (19.5 — 22.5 °C.) and at a range higher (33.1 — 36.2 °C.). The upper and lower limits of lethal temperatures are also determined.

4. The rate of oxygen consumption of the prawn is not directly affected by changes in hydrogen-ion cocentration of the medium.

5. The rate of oxygen consumption is unaffected by changes in oxygen content of the water between 7.74 and 3.72 ml./L. A further decrease in the oxygen tension lessens the rate considerably.

6. The results obtained are discussed in the light of previous work on the subject.

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PART III

SEASONAL FLUCTUATIONS IN THE FAT CONTENT OF THE PRAWN *PENAEUS INDICUS*

III. SEASONAL FLUCTUATIONS IN THE FAT CONTENT OF THE PRAWN *PENAEUS INDICUS*

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PART III

SEASONAL FLUCTUATIONS IN THE FAT CONTENT OF THE PRAWN *PENAEUS INDICUS*

INTRODUCTION

Although in recent years considerable attention has been given to estimation of fat values of fishes (See Hickling, 1947; Patakoot *et al*, 1950; Sekharan, 1950; Chidambaram *et al*, 1952; Venkataraman and Chari, 1953) the commercially important penaeid prawns, with a few exceptions, have not been studied from the above point of view. From a study of the chemical composition of prawns, Appanna and Devadatta (1942), Chari (1948) and Gopalakrishnan (1951) have shown that as in fishes fat constitutes a food reserve along with proteins. Further, it has been shown by Koyama (1929) that doubly refracting fat is present in all organs except the heart and blood vessels of *Penaeus japonicus.* It is generally known that the fat content of animals is subject to periodic quantitative fluctuations in the tissues. But the workers in the field are not agreed as to the factors which cause such changes. This is especially so in the case of fishes, which have been studied in regard to the seasonal fluctuations in stored fat and various suggestions have been made as to contributing causes for such changes. Temperature of the external medium was shown to influence the fat content of some fishes by Johnstone (1917), but this view has not been found valid by Dixon (1937) for sprats from Norway. Age is another factor suggested by Bruce (1924), Lovern (1938) and Wilson (1939). Johnstone (1917), Greene (1919) and Bull (1928) thought that the maturation of gonads and spawning season have a direct influence in the amount of fat present. Channon and Saby (1932), Lovern and Wood (1937), Dixon (1937), Wilson (1939) and Hickling (1947) were able to demonstrate relations between food and feeding on the one hand and the quantitative changes in fat content on the other. In view of the lack of information as to the fluctuations, if any, in the fat content of prawns and to see if the same causes that account for such changes in the fishes also operate here, the fat contents of the prawn *Penaeus indicus*, collected in different months of the year, are estimated and studied in the present work.

MATERIAL AND METHODS

The prawns used in the following study were collected from the sea and the brackish water areas of the Cooum river. Fat estimations of different length groups were made during the course of a year. For the purpose of this study the prawns studied were divided into four different length groups as follows.

Length group I. Length below 11 cms.

Length group II. Length between 11 and 14 cms.

Length group III. Length between 14 and 17 cms.

Length group IV. Length between 17 and 20 cms.

Chemical extractions of fat were made using both male and female prawns of different sizes collected from both the localities mentioned above. For each extraction, the following procedure was adopted. About three or four speciments belonging to the same sex, length group and locality were isolated from the collections obtained from the nets. The whole prawn except the stomach and its contents was used for the estimation. The specimens chosen were cut into small pieces and then minced thoroughly in a meat mincer, until a pulp of the tissue was obtained. The whole quantity of minced tissue was mixed in a clean porcelain mortar and enough quantity was taken in a glass dish and weighed. This was then dried in a constant temperature apparatus at 105 °C. until all traces of moisture disappeared. For the estimation of fat present in this dried tissue, a Soxhlet extraction apparatus was used. The solvent in the flask, ether sulphuric, was heated by an electric heater provided with a regulator, and ice-cooled water was circulated in the condenser of the apparatus. In the first few experiments the process of extraction was carried on till the weight of the flask with the extract was constant for successive periods. Later it was noticed from the results of the first experiments that continuous extraction for seven hours was enough to isolate all the fat present. That this is so was confirmed by extraction of the residual tissue present in the thimble to detect the presence of any fat left unextracted and in all cases the results were negative. Two or three extractors were used simultaneously whenever necessary.

It is known that the fat thus extracted may contain slight amounts of other substances dissolving in ether (Sherman. 1941). However, ether is the best solvent for fat and hence some authors designate the material extracted as 'ether extract'

rather than as fat. The term 'fat' itself is used in this paper, as the amount of other substances that may be present along with it are considered negligible.

The histological technique as adopted by Wilson (1939) for studying the distribution of fat in fishes was tried, but was not successful, as such well marked regions of fat storage could not be made out in the sections of prawns.

LENGTH GROUPS AND THEIR FAT VALUES IN DIFFERENT MONTHS

FAT CONTENT OF MARINE PRAWNS.

Prawns of all the four different length groups were available from the fishermen's nets during the major part of the year. The fat values of prawns of the different length groups are recorded in Tables I, II, III and IV.

(a) Length group I.

The experimental results are given in Table I and Fig. 1 and 2. In Fig. 1 the fat values obtained for males during the various months are plotted. The percentage is seen to be low in August and continues to be so till November, after which there is a rise during December. In March the value decreases and only slightly higher percentages are noted during the following months of April and May. In the female specimens (Fig. 2) the fat values are more or less similar to those obtained for the males. The fat content percentage is low in August, but rises steadily till December when the peak value of about 1.3% is reached. A fall in percentage content occurs by February after which the value is more or less stationary. Minor variations from the normal do not affect the overall trend of the graph.

(b) Length group II.

Similar fat estimations were made for prawns of length between 11 and 14 cms. Sexes were examined separately and extractions were made during all the months except September and February. The number of specimens studied and their average fat percentages are shown in Table II and Figs. 1 and 2. The amount of fat present in prawns of this length group show that the percentage values correspond with those for the smallest length group. It will be noted from the Table and Figs. that the maximum amount of fat occurs during the months of December and January.

TABLE I

PERCENTAGE OF FAT (ON FRESH BASIS) IN MARINE *PENAEUS INDICUS* OF LENGTH GROUP I

Month	Sex	No. of specimens studied	Percentage of fat (by weight)
August	Male	12	0·97
	Female	12	0·93
September	Male	8	0·90
	Female	10	1·02
October	Male	10	0·93
	Female	10	0·99
November	Male	8	0·88
	Female	10	1·06
December	Male	8	1·22
	Female	12	1·27
January	Male	...	...
	Female	...	...
February	Male	14	1·15
	Female	12	0·92
March	Male	12	0·90
	Female	12	1·01
April	Male	10	1.03
	Female	10	1.08
May	Male	8	1.13
	Female	6	1.16
June	Male	12	1·01
	Female	12	1·05
July	Male	8	1·08
	Female	8	0·97

TABLE II

PERCENTAGE OF FAT (ON FRESH BASIS) IN MARINE *PENAEUS INDICUS* OF LENGTH GROUP II

Month	Sex	No. of specimens studied	Percentage of fat (by weight)
August	Male	8	0·93
	Female	10	0·99
September	Male	...	...
	Female	...	...
October	Male	10	1·04
	Female	6	0·88
November	Males	10	0·97
	Females	8	0·84
December	Male	6	1·24
	Female	8	1·33
January	Male	14	1·32
	Female	14	1.13
February	Male	...	...
	Female	...	...
March	Male	10	1·14
	Female	10	0·87
April	Male	10	1·05
	Female	10	0·88
May	Male	10	0·95
	Female	10	1·09
June	Male	6	1·06
	Female	8	1·11
July	Male	10	1·12
	Female	10	1·05

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TABLE III

PERCENTAGE OF FAT (ON FRESH BASIS) IN MARINE *PENAEUS INDICUS* OF LENGTH GROUP III

Month	Sex	No. of specimens studied	Percentage of fat (by weight)
August	Male	8	1·03
	Female	10	0·95
September	Male	6	0·85
	Female	6	0·80
October	Male	6	1·10
	Female	8	1·06
November	Male	4	1·36
	Female	8	1·43
December	Male	6	1·38
	Female	6	1·44
January	Male	...	...
	Female	6	1·14
February	Male	6	1·18
	Female	12	1·07
March	Male	8	0·99
	Female	8	0·94
April	Male	10	0·95
	Female	8	1·01
May	Male	8	0·90
	Female	8	0·96
June	Male	8	0·88
	Female	8	0·92
July	Male	6	0·97
	Female	6	0·90

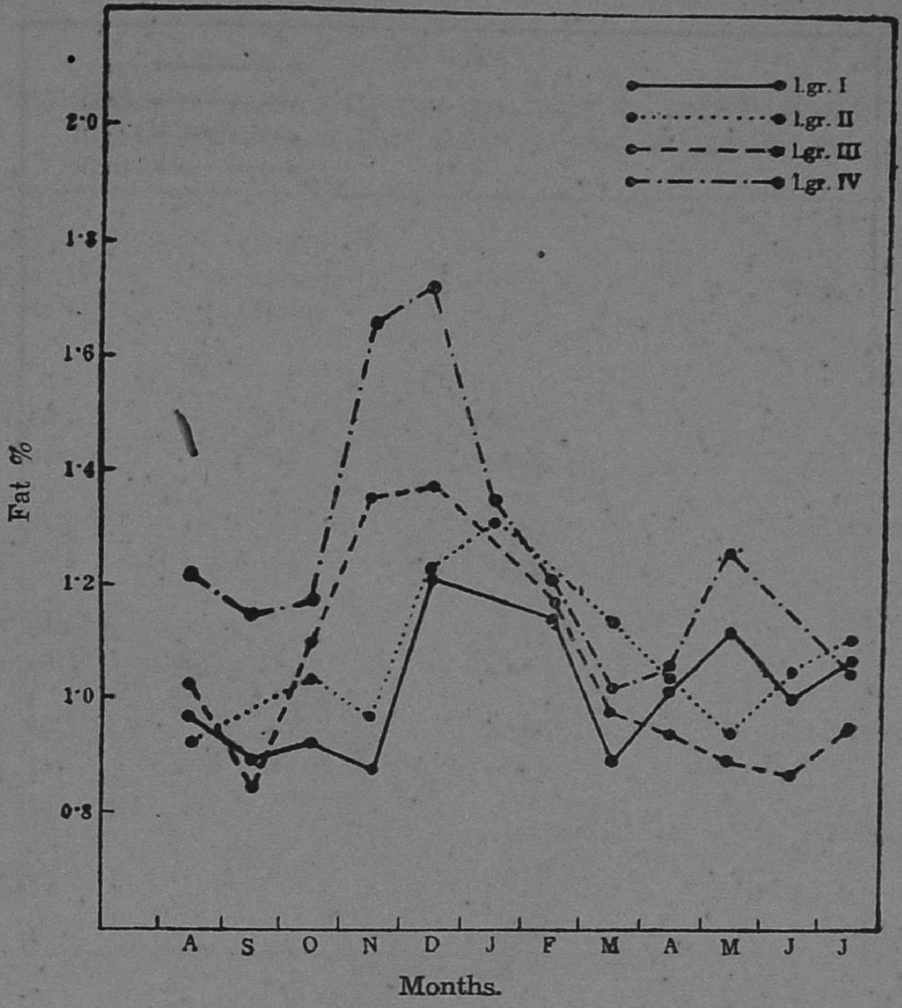

Fig. 1.

Curves illustrating the percentage fat content of the four length groups of *Penaeus indicus* (males; marine) in different months.

(c) Length group III.

Results obtained from regular monthly fat estimations of prawns of this length group are presented in Table III. As in the smaller specimens, seasonal fluctuations in the fat content are obvious in these prawns also and it will be noted that the maximum amount of fat is present during the months of November and December, in the males as well as in the females (Figs. 1 and 2). It may also be mentioned that the percentage values are approximately the same for the two sexes.

(d) Length group IV.

This length group consists of prawns of the biggest size usually obtainable from the fishermen's nets and they were available for the present study during all the months except

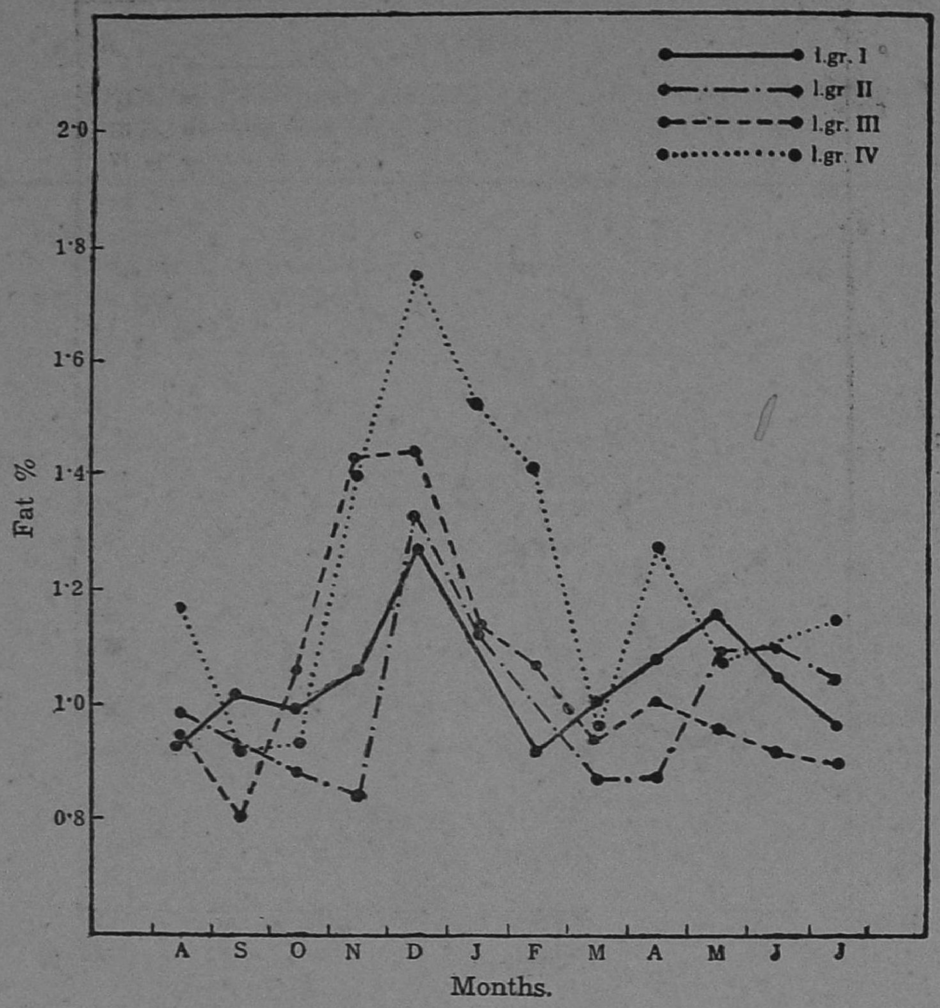

Fig. 2.

Curves illustrating the percentage fat content of the four length groups of *Penaeus indicus* (females; marine) in different months.

June. The average fat contents of these large prawns during the various months are shown in Table IV and also Figs. 1 and 2. It appears that the percentage of fat present in prawns of this length group is comparatively more than in the smaller forms. For the males, during the months of August, September and October the fat values are low, but in November and December very high percentages are obtained. After December, the value decreases till March and further fluctuations are not pronounced. In the female specimens also the fat value is found to be highest during December, the percentage being about 1.8.

It is interesting to note from the results obtained that in all the length groups studied the highest fat values are recorded

TABLE IV

PERCENTAGE OF FAT (ON FRESH BASIS) IN MARINE *PENAEUS INDICUS* OF LENGTH GROUP IV

Month	Sex	No. of specimens studied	Percentage of fat (by weight)
August	Male	10	1·22
	Female	10	1·17
September	Male	8	1·15
	Female	6	0·92
October	Male	6	1·17
	Female	4	0·93
November	Male	6	1·66
	Female	6	1 40
December	Male	12	1·73
	Female	8	1·75
January	Male	10	1·35
	Female	6	1·53
February	Male	8	1·22
	Female	6	1·41
March	Male	8	1 03
	Female	8	0·96
April	Male	8	1·07
	Female	12	1·28
May	Male	6	1·27
	Female	6	1·08
June	Male	...	...
	Female	...	...
July	Male	8	1·06
	Female	8	1·15

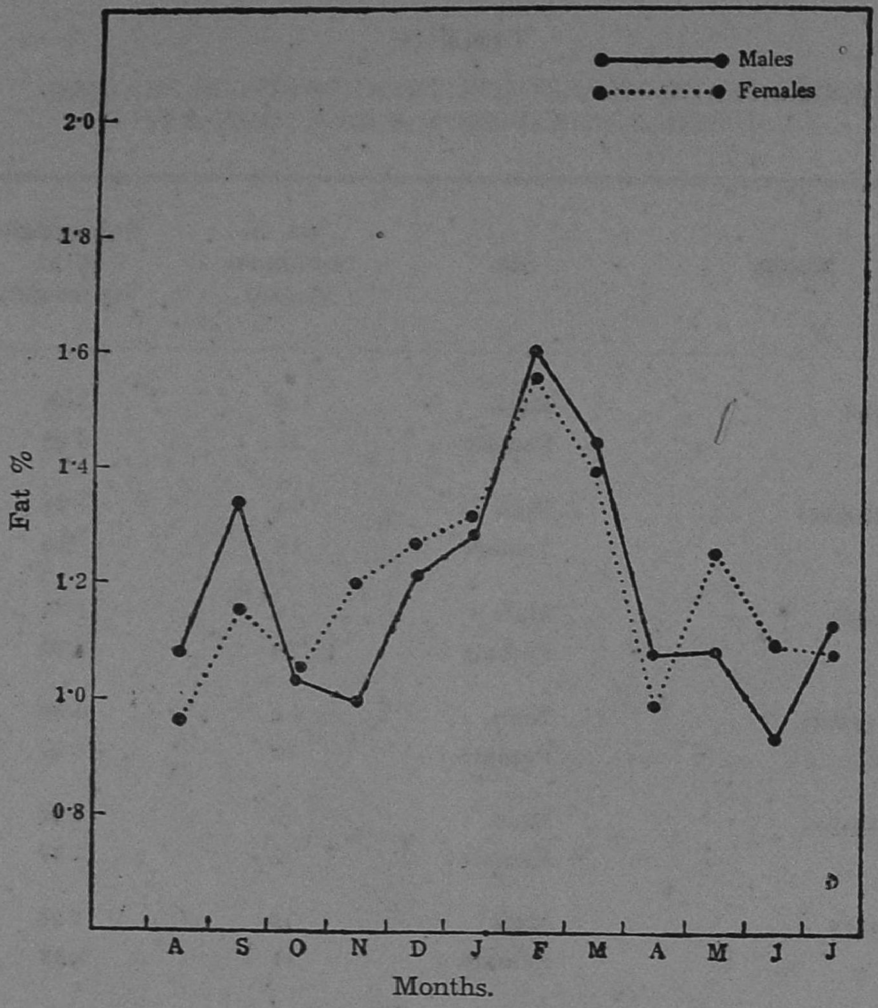

Fig. 3.

Curves illustrating the percentage fat content of brackish water *Penaeus indicus* (both sexes; length gr. I) in different months.

during the season comprising November, December and January and that the seasonal fluctuations are more or less similar for prawns of both sexes.

Fat content of prawns collected from the Cooum river

Only smaller specimens were available from this brackish water area and hence all the speciments used for the fat estimations were young forms, of length group I. Fat estimations were made during all the months, taking the sexes separately. The number of prawns used and the results obtained from the extractions are given in Table V. The graph (Fig. 3) prepared from the average fat values shows that there is great similarity in the amount of fat stored in prawns of both sexes during most of the months. Slight differences are noticed during September, November, May, and June, but the significance of these

TABLE V

PERCENTAGE OF FAT (ON FRESH BASIS) IN *PENAEUS INDICUS* COLLECTED FROM THE COOUM RIVER

Month	Sex	No. of specimens studied	Percentage of fat (by weight)
August	Male	6	1·08
	Female	10	0·96
September	Male	14	1·34
	Female	28	1·15
October	Male	16	1·04
	Female	28	1·05
November	Male	12	0·99
	Female	30	1·20
December	Male	12	1·22
	Female	32	1·27
January	Male	14	1·29
	Female	14	1·32
February	Male	16	1·61
	Female	16	1·56
March	Male	14	1·45
	Female	16	1·39
April	Male	14	1·08
	Female	14	0·93
May	Male	10	1·09
	Female	14	1·26
June	Male	10	0·94
	Female	10	1·10
July	Male	12	1·13
	Female	10	1·09

differences is not clear. In the male specimens there is a decrease in fat content from September to November, after which higher values are obtained in January, February and March. It appears that in the case of the female specimens, the fat content is low in August, but increases gradually and steadily till February when the highest value is reached. Afterwards there is a decrease up to April and although there is a slight rise in May, the value is lower in June and July. It will be noted that in both the sexes the highest fat percentage is found during February.

During certain months prawns of length group II were obtained in the collections, but they were not used for the present study as such forms were not available throughout the year.

Distribution of Fat in the Prawn

Storage of fat in *Penaeus indicus* is confined mainly to the hepatopancreas, gonads and certain muscles. Sections of these tissues cut with a freezing microtome were stained in Sudan III and examined under the microscope to study the fat storage. It is known that in decapods the hepatopancreas is the principal storage organ for the reserve fat (Yonge, 1924) and that the amount of fat stored in the organ is closely related with the moulting (Paul and Sharpe, 1919; Yonge, 1924). In a section of the hepatopancreas of *Penaeus indicus* fat globules are observed in the tubules and in the small vacuoles present in the absorptive cells. They are never seen in the secretory cells of the digestive gland.

Sections of the ovary of the prawn show the presence of fat globules deposited in the "nutritive cells" surrounding the radially arranged strings of ova. The amount of fat present in the testis is very little and is seen as small droplets in the central lumen of the lobules.

The abdominal muscles of the prawn also contain small quantities of fat, the amount present being much less compared to that in fishes. A transverse slice through the anterior part of the abdomen of the animal is taken as typical for the amount of fat present in the muscles. The flexor muscles of the abdomen show the presence of very small fat globules. These globules are deposited in between the muscle fibres and are in the form of fat storing cells provided with fine enclosing membranes. Fat droplets are usually seen in the fibres of the extensor muscles of the abdomen also. In addition to these, small fat globules occur in between the abdominal cuticle and muscles.

Food of Penaeus indicus

An analysis of the stomach contents of *Penaeus indicus* (Gopalakrishnan, 1952) shows that both vegetable and animal matter are taken right throughout the year and the animal matter in which crustacean and non-crustacean items can be recognised, varies in composition. The fluctuations in the relative amounts of non-crustacean and crustacean constituents of the diet were followed during the different months of the year (Gopalakrishnan, loc. cit.).

Discussion

It has been shown that in fishes the amount of fat stored in the tissue depends on the quantity and quality of food taken by the fish (Channon and Saby, 1932; Lovern and Wood, 1937; Dixon, 1937; Hickling, 1947; Sekharan, 1950; Chidambaram *et al*, 1952). In the present study on the seasonal fluctuations in the fat content of the prawn, it is noticed that the maximum percentages of fat occur during the months of November, December and January. The analysis of stomach contents shows that a greater proportion of non-crustacean food elements, such as lamellibranchs, fish larvae etc., are consumed during the above months. This suggests that non-crustacean food elements are productive of more fat. It is probable that in an animal like the prawn, whose diet includes different types of food materials, the composition may affect the fat income to a greater extent than in fishes, in which usually there is not such variety of food items. It has been shown that the fluctuations in the amount of stored fat can be related to the changes in the composition of the diet of fishes (See Hickling, 1947 and Sekharan, 1950). When the fish consumes vegetable matter and non-crustacean animals (excepting teleosts), the quantity of fat present is much less than when it consumes crustacean and teleostean food items (Sekharan, unpublished thesis). It is possible that further detailed analysis of the stomach contents of the prawn and experiments on its diet may show a similar relation between the food and fat content of the prawn.

It will be noted from the data presented that prawns of the highest length group are found to have more percentage of fat than those of shorter length groups. This increase in the amount of fat stored may be attributed to their greater intake of food. Similar increases in fat contents due to length and also age has been reported in some fishes also (Bruce, 1924; Lovern, 1938; Wilson, 1939).

It is evident that the hepatopancreas, the female gonad and the abdominal reflexor and extensor muscles are the chief organs of fat storage in *Penaeus indicus*. It is possible that these organs show a fluctuation in fat storage corresponding to the seasonal changes in the total fat content of the prawn. Detailed analysis of the different parts of the prawn, viz., liver, gonad and muscles, during the different seasons of the year and in the immature and mature forms, may reveal an intricate adjustment of the total amount of fat, not unlike what is found in fish (Ramaswamy, unpublished).

Summary

1. The seasonal fluctuations in the fat content of the prawn *Penaeus indicus* are studied.

2. Four different length groups were chosen and the fluctuations in the fat content during the course of a year estimated. It is seen that in the highest length group (Group IV) the percentage of fat is greatest and that the maximum percentage of fat occurs during the period November—January in all the length groups.

3. The percentage of fat stored appears to be nearly the same in prawns of both sexes.

4. The relationship between the fat content and feeding habits of the prawn is surveyed.

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